# TOWNSEND
### and
# TOWNSEND
### and
# CREW
LLP

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Eighth Floor
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5 January 2004

*VIA EXPRESS MAIL, WITH RETURN POSTCARD ENCLOSED*

PCT International Application Processing Div.
USPTO International Division
Assistant Commissioner for Patents
Mail Stop PCT
PO Box 1450
Alexandria, VA 22313-1450

Re:  International Application No. PCT/US03/17725
Title: METHODS OF DIAGNOSING & TREATING DIABETES AND INSULIN RESISTANCE
Applicant: METABOLEX, INC. *et al.*
International Filing Date: 4 June 2003
Express Mail Label No.: EV 330 850 185 US
Date of Mailing: 5 January 2004
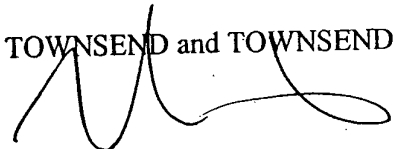Our File No.: 16325-139PC

Dear Officer:

Enclosed are the Chapter II Demand and four (4) substitute specification pages 4, 22, 23 and 42 submitted as an Article 34 Amendment for the above-referenced patent application. The only changes were insertions of SEQ ID:NOs. and corrections of typographical errors that do not include matter which go beyond the disclosure in the international application as filed.

Thank you for your attention to this matter.

Respectfully submitted,

TOWNSEND and TOWNSEND and CREW LLP

Matthew E. Hinsch
Reg. No. 47,651

MEH/nan
Enclosures:     Chapter II Demand
Four (4) substitute specification pages 4, 22, 23 and 42
Twenty (20) pages of Sequence Listing
Diskette and Statement
Transmittal Letter and Postcard

60109699 v1

*The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are competent,*
*with the one chosen by the applican___ full name or two-letter code of that Authority may be indicated by the applicant on the line below:*

IPEA/ US

# PCT

## DEMAND

| CHAPTER II |
|---|

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of
international preliminary examination according to the Patent Cooperation Treaty and
hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

| Identification of IPEA | Date of receipt of DEMAND |
|---|---|

| Box No. I    IDENTIFICATION OF THE INTERNATIONAL APPLICATION | Applicant's or agent's file reference<br>16325-139PC |
|---|---|

| International application No.<br><br>PCT/US03/17725 | International filing date *(day/month/year)*<br><br>04 June 2003 (04.06.03) | (Earliest) Priority date *(day/month/year)*<br><br>04 June 2002 (04.06.02) |
|---|---|---|

**Title of invention**

METHODS OF DIAGNOSING & TREATING DIABETES AND INSULIN RESISTANCE

**Box No. II    APPLICANT(S)**

| Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*<br><br>METABOLEX, INC.<br>3876 Bay Center Place<br>Hayward, California 94545<br>United States of America | Telephone No.:<br>510.293.8800 |
|---|---|
| | Facsimile No.:<br>510.293.9090 |
| | Teleprinter No.: |
| | Applicant's registration No. with the Office |

| State *(that is, country)* of nationality:<br><br>US | State *(that is, country)* of residence:<br><br>US |
|---|---|

| Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*<br><br>ALLAN, Bernard<br>940 Guerrero Street<br>San Francisco, California 94110<br>United States of America | |
|---|---|

| State *(that is, country)* of nationality:<br><br>IE | State *(that is, country)* of residence:<br><br>US |
|---|---|

| Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*<br><br>GREGOIRE, Francine<br>1044 Carol Lane<br>Lafayette, California 94549<br>United States of America | |
|---|---|

| State *(that is, country)* of nationality:<br><br>BE | State *(that is, country)* of residence:<br><br>US |
|---|---|

☒ Further applicants are indicated on a continuation sheet.

*See Notes to the demand form*

Form PCT/IPEA/401 (continuation sheet) (March 2001; reprint January 2003)

Continuation of Box No. I   APPLICANT(S)

*If none of the following sub-boxes is used, this sheet should not be included in the demand.*

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LAVAN, Brian
2020 Lawton Street
San Francisco, California 94122
United States of America

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| GB | US |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

MOODIE, Shonna
2091 Golden Gate
San Francisco, California 94115
United States of America

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| GB | US |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| | |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| | |

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

| State *(that is, country)* of nationality: | State *(that is, country)* of residence: |
|---|---|
| | |

*See Notes to the demand form*

Form PCT/IPEA/401 (continuation sheet) (March 2001; reprint January 2003)

| Box No. III | AGENT OR ●●MON REPRESENTATIVE; OR ADDRESS F●● ●ORRESPONDENCE |

The following person is    ☒ agent    ☐ common representative

and   ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

     ☐ is hereby appointed and any earlier appointment of (an) agent(s)/common representative is hereby revoked.

     ☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

| Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)* | Telephone No.: 415.576.0200 |
| --- | --- |
| HINSCH, Matthew, E.<br>TOWNSEND AND TOWNSEND AND CREW LLP<br>Two Embarcadero Center, 8th Floor<br>San Francisco, California 94111-3834<br>United States of America | Facsimile No.: 415.576.0300 |
| | Teleprinter No.: |
| | Agent's registration No. with the Office 47,651 |

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

| Box No. IV | BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION |

**Statement concerning amendments:** *

1. The applicant wishes the international preliminary examination to start on the basis of:

     ☒ the international application as originally filed

   the description    ☐ as originally filed
                         ☒ as amended under Article 34

   the claims    ☒ as originally filed
                   ☐ as amended under Article 19 (together with any accompanying statement)
                   ☐ as amended under Article 34

   the drawings    ☒ as originally filed
                      ☐ as amended under Article 34

2.   ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3.   ☐ The applicant wishes the start of the international preliminary examination to be postponed until the expiration of 20 months from the priority date unless the International Preliminary Examining Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

\* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

**Language for the purposes of international preliminary examination:**    **ENGLISH** ...................................................

     ☒ which is the language in which the international application was filed.
     ☐ which is the language of a translation furnished for the purposes of international search.
     ☐ which is the language of publication of the international application.
     ☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

| Box No. V | ELECTION OF STATES |

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

     excluding the following States which the applicant wishes **not to elect:**

*See Notes to the demand form*

International application No.

**PCT/US03/17725**

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**Box No. VI     CHECK LI**⬤ ⬤

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

| | | For International Preliminary Examining Authority use only | |
|---|---|---|---|
| | | received | not received |

1. translation of international application          :          sheets       ☐ ☐

2. amendments under Article 34          :     4   sheets       ☐ ☐

3. copy (or, where required, translation) of amendments under Article 19          :          sheets       ☐ ☐

4. copy (or, where required, translation) of statement under Article 19          :          ‹ sheets       ☐ ☐

5. letter          :     1   sheet       ☐ ☐

6. other (specify)          :          sheets       ☐ ☐

---

The demand is also accompanied by the item (s) marked below:

1. ☒ fee calculation sheet

2. ☐ original separate signed power of attorney

3. ☐ original general power of attorney;

4. ☐ copy of general power of attorney; reference number, if any:

5. ☐ statement explaining lack of signature

6. ☒ sequence listing in computer readable form

7. ☐ tables in computer readable form related to sequence listings

8. ☒ other (specify) Transmittal Letter; Article 34 Amendment with four (4) substitute specification pages 4, 22, 23 and 42; Twenty (20) pages of Sequence Listing, Statement and Diskette; Postcard

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**Box No. VII     SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE**

Next to each signature, indicate the name of the person signing and the capacity in which the person  signs (if such capacity is not obvious from reading the demand).

X _____

Matthew E. Hinsch
TOWNSEND AND TOWNSEND AND CREW LLP
USPTO Reg. No.: 47,651
Applicants' Agent

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For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:

2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):

3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply.          ☐ The applicant has been informed accordingly.

4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.

5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

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For International Bureau use only

Demand received from IPEA on:

See Notes to the demand form

# PCT

## FEE CALCULATION SHEET

### Annex to the Demand

For International Preliminary Examining Authority use only

| International application No. | PCT/US03/17725 | |
|---|---|---|
| Applicant's or agent's file reference | 16325-139PC | Date stamp of the IPEA |

Applicant

METABOLEX, INC. *et al.*

## CALCULATION OF PRESCRIBED FEES

1. Preliminary examination fee ................................. | 490.00 | **P**

2. Handling fee (*Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.*) ....................................... | 172.00 | **H**

3. Total of prescribed fees
   Add the amounts entered at P and H
   and enter total in the TOTAL box ..................... | 662.00 |
   | TOTAL |

## MODE OF PAYMENT

☒ authorization to charge deposit account with the IPEA (see below)

☐ cheque

☐ postal money order

☐ bank draft

☐ cash

☐ revenue stamps

☐ coupons

☐ other (*specify*):

## AUTHORIZATION TO CHARGE (OR CREDIT) DEPOSIT ACCOUNT
(*This mode of payment may not be available at all IPEAs*)

The IPEA/ __US__

☒ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ (*this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit*) is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

| 20-1430 | 5 January 2004 | Signature   Matthew B. Hinsch |
|---|---|---|
| Deposit Account Number | Date (*day/month/year*) | *See Notes to the fee calculation sheet* |

Form PCT/IPEA/401 (Annex) (July 1998; reprint July 1999)
(60109700 v1)

[16]    In some embodiments, the detecting step comprises quantifying mRNA encoding the polypeptide. In some embodiments, the mRNA is reverse transcribed and amplified in a polymerase chain reaction.

[17]    In some embodiments, the sample is a blood, urine or tissue sample.

[18]    The present invention also provides isolated nucleic acids that hybridize under stringent conditions to a polynucleotide encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

[19]    In some embodiments, the nucleic acid is selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13

[20]    The present invention also provides an expression cassette comprising a heterologous promoter operably linked to a polynucleotide that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

[21]    The present invention also provides host cells transfected with a polynucleotide that hybridizes under stringent conditions to a nucleic acid encoding a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14. In some embodiments, the host cell is a human cell. In some embodiments, the host cell is a bacterium.

[22]    The present invention also provides isolated polypeptides comprising an amino acid sequence at least 70% identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14. In some embodiments, the polypeptide is SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

DEFINITIONS

[23]    "Insulin sensitivity" refers to the ability of a cell or tissue to respond to insulin. Responses include, e.g., glucose uptake of a cell or tissue in response to insulin stimulation. Sensitivity can be determined at an organismal, tissue or cellular level. For example, blood or urine glucose levels following a glucose tolerance test are indicative of insulin sensitivity. Other methods of measuring insulin sensitivity include, e.g., measuring

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## C. Standard Protein Separation Techniques For Purifying Proteins

### 1. Solubility Fractionation

[82]     Often as an initial step, and if the protein mixture is complex, an initial salt fractionation can separate many of the unwanted host cell proteins (or proteins derived from the cell culture media) from the recombinant protein of interest. The preferred salt is ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the amount of water in the protein mixture. Proteins then precipitate on the basis of their solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower ammonium sulfate concentrations. A typical protocol is to add saturated ammonium sulfate to a protein solution so that the resultant ammonium sulfate concentration is between 20-30%. This will precipitate the most hydrophobic proteins. The precipitate is discarded (unless the protein of interest is hydrophobic) and ammonium sulfate is added to the supernatant to a concentration known to precipitate the protein of interest. The precipitate is then solubilized in buffer and the excess salt removed if necessary, through either dialysis or diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol precipitation, are well known to those of skill in the art and can be used to fractionate complex protein mixtures.

### 2. Size Differential Filtration

[83]     Based on a calculated molecular weight, a protein of greater and lesser size can be isolated using ultrafiltration through membranes of different pore sizes (for example, Amicon or Millipore membranes). As a first step, the protein mixture is ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of the protein of interest. The recombinant protein will pass through the membrane into the filtrate. The filtrate can then be chromatographed as described below.

### 3. Column Chromatography

[84]     The proteins of interest can also be separated from other proteins on the basis of their size, net surface charge, hydrophobicity and affinity for ligands. In addition, antibodies raised against proteins can be conjugated to column matrices and the proteins immunopurified. All of these methods are well known in the art.

[85]     Immunoaffinity chromatography using antibodies raised to a variety of affinity tags such as hemagglutinin (HA), FLAG, Xpress, Myc, hexahistidine (SEQ ID NO:15) (His), glutathione S transferase (GST) and the like can be used to purify

polypeptides. The His tag will also act as a chelating agent for certain metals (e.g., Ni) and thus the metals can ● be used to purify His-containing poly●tides. After purification, the tag is optionally removed by specific proteolytic cleavage.

[86]    It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (e.g., Pharmacia Biotech).

## IV.    DETECTION OF POLYNUCLEOTIDES OF THE INVENTION

[87]    Those of skill in the art will recognize that detection of expression of polynucleotides and polypeptides of the invention has many uses. For example, as discussed herein, detection of levels of polynucleotides and polypeptides of the invention in a patient is useful for diagnosing diabetes or a predisposition for at least some of the pathological effects of diabetes. Moreover, detection of gene expression is useful to identify modulators of expression of polynucleotides and polypeptides of the invention.

[88]    A variety of methods of specific DNA and RNA measurement that use nucleic acid hybridization techniques are known to those of skill in the art (see, Sambrook, supra). Some methods involve an electrophoretic separation (e.g., Southern blot for detecting DNA, and Northern blot for detecting RNA), but measurement of DNA and RNA can also be carried out in the absence of electrophoretic separation (e.g., by dot blot). Southern blot of genomic DNA (e.g., from a human) can be used for screening for restriction fragment length polymorphism (RFLP) to detect the presence of a genetic disorder affecting a polypeptide of the invention.

[89]    The selection of a nucleic acid hybridization format is not critical. A variety of nucleic acid hybridization formats are known to those skilled in the art. For example, common formats include sandwich assays and competition or displacement assays. Hybridization techniques are generally described in Hames and Higgins Nucleic Acid Hybridization, A Practical Approach, IRL Press (1985); Gall and Pardue, Proc. Natl. Acad. Sci. U.S.A., 63:378-383 (1969); and John et al. Nature, 223:582-587 (1969).

[90]    Detection of a hybridization complex may require the binding of a signal-generating complex to a duplex of target and probe polynucleotides or nucleic acids. Typically, such binding occurs through ligand and anti-ligand interactions as between a ligand-conjugated probe and an anti-ligand conjugated with a signal. The binding of the signal generation complex is also readily amenable to accelerations by exposure to ultrasonic energy.

[162] Similarly, any haptenic or antigenic compound can be used in combination with appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and the tag binder is a second antibody that recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs, such as agonists and antagonists of cell membrane receptors (e.g., cell receptor-ligand interactions such as transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies, the cadherin family, the integrin family, the selectin family, and the like; see, e.g., Pigott & Power, The Adhesion Molecule Facts Book I (1993)). Similarly, toxins and venoms, viral epitopes, hormones (e.g., opiates, steroids, etc.), intracellular receptors (e.g., which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

[163] Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

[164] Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly-Gly sequences of between about 5 and 200 amino acids (SEQ ID NO:16). Such flexible linkers are known to those of skill in the art. For example, poly(ethylene glycol) linkers are available from Shearwater Polymers, Inc., Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

[165] Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by exposing all or a portion of the substrate to a chemical reagent that fixes a chemical group to the surface that is reactive with a portion of the tag binder. For example, groups that are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer

January 5, 2004

*VIA EXPRESS MAIL*

Special Programs Examiner Sue Wolski
Mail Stop PCT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Re:  International Application No. PCT/US03/17725
filed 04 June 2003
Applicant(s):  Metabolex, Inc. *et al.*
Inventor(s):  Allan et al.
Title:  Methods of Diagnosing & Treating Diabetes and Insulin Resistance
Our Ref.:  16325-139PC

Dear Special Programs Examiner Wolski:

In order to comply with the WIPO Standard ST.25 requirements for the Sequence Listing, please find enclosed the Sequence Listing and disk at this time.

It is hereby stated that "the information recorded on the computer readable form is identical to the written sequence listing" and does not include matter which goes beyond the disclosure in the international application as filed.

Sincerely,

Matthew E. Hinsch, Esq.

MEH:dmw
Enclosures
60072934 v1

<110> Allan, Be●●d
     Gregoire, ●●ncine
     Lavan, Brian
     Moodie, Shonna
     Metabolex, Inc.

<120> Methods of Diagnosing & Treating Diabetes and Insulin, Resistance

<130> 016325-013900PC

<140> WO PCT/US03/17725
<141> 2003-06-04

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<210> 2
<211> 349
<212> PRT
<213> Homo sapiens

<220>
<223> human connective tissue growth factor (CTGF)

<400> 2
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 1               5                  10                  15

Leu Ala Leu Cys Ser Arg Pro Ala Val Gly Gln Asn Cys Ser Gly Pro
            20                  25                  30

Cys Arg Cys Pro Asp Glu Pro Ala Pro Arg Cys Pro Ala Gly Val Ser
        35                  40                  45

Leu Val Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Lys Gln Leu
    50                  55                  60

Gly Glu Leu Cys Thr Glu Arg Asp Pro Cys Asp Pro His Lys Gly Leu
65                  70                  75                  80

Phe Cys Asp Phe Gly Ser Pro Ala Asn Arg Lys Ile Gly Val Cys Thr
                85                  90                  95

Ala Lys Asp Gly Ala Pro Cys Ile Phe Gly Gly Thr Val Tyr Arg Ser
            100                 105                 110

Gly Glu Ser Phe Gln Ser Ser Cys Lys Tyr Gln Cys Thr Cys Leu Asp
        115                 120                 125

Gly Ala Val Gly Cys Met Pro Leu Cys Ser Met Asp Val Arg Leu Pro
    130                 135                 140

Ser Pro Asp Cys Pro Phe Pro Arg Arg Val Lys Leu Pro Gly Lys Cys
145                 150                 155                 160

Cys Glu Glu Trp Val Cys Asp Glu Pro Lys Asp Gln Thr Val Val Gly
                165                 170                 175
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2

Pro Ala Leu Ala Ala Tyr Arg Leu Glu Asp Thr Phe Gly Pro Asp Pro
        180                 185                 190

Thr Met Ile Arg ● Asn Cys Leu Val Gln Thr Thr G● rp Ser Ala
        195                200              20●

Cys Ser Lys Thr Cys Gly Met Gly Ile Ser Thr Arg Val Thr Asn Asp
      210              215             220

Asn Ala Ser Cys Arg Leu Glu Lys Gln Ser Arg Leu Cys Met Val Arg
225              230            235            240

Pro Cys Glu Ala Asp Leu Glu Glu Asn Ile Lys Lys Gly Lys Lys Cys
        245              250            255

Ile Arg Thr Pro Lys Ile Ser Lys Pro Ile Lys Phe Glu Leu Ser Gly
        260              265            270

Cys Thr Ser Met Lys Thr Tyr Arg Ala Lys Phe Cys Gly Val Cys Thr
      275            280            285

Asp Gly Arg Cys Cys Thr Pro His Arg Thr Thr Thr Leu Pro Val Glu
      290            295            300

Phe Lys Cys Pro Asp Gly Glu Val Met Lys Lys Asn Met Met Phe Ile
305              310            315            320

Lys Thr Cys Ala Cys His Tyr Asn Cys Pro Gly Asp Asn Asp Ile Phe
        325              330            335

Glu Ser Leu Tyr Tyr Arg Lys Met Tyr Gly Asp Met Ala
        340              345


<210> 3
<211> 2330
<212> DNA
<213> Mus musculus

<220>
<223> mouse connective tissue growth factor (CTGF)
     homolog cDNA

<220>
<221> CDS
<222> (204)..(1250)
<223> CTGF homolog

<400> 3
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<210> 4
<211> 348
<212> PRT
<213> Mus musculus

<220>
<223> mouse connective tissue growth factor (CTGF)
      homolog

<400> 4
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Met Leu Ala Ser Val Ala Gly Pro Ile Ser Leu Ala Leu Val Leu Leu
  1               5                  10                  15

Ala Leu Cys Thr Arg Pro Ala Thr Gly Gln Asp Cys Ser Ala Gln Cys
                 20                  25                  30

Gln Cys Ala Ala Glu Ala Ala Pro His Cys Pro Ala Gly Val Ser Leu
             35                  40                  45

Val Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Lys Gln Leu Gly
         50                  55                  60

Glu Leu Cys Thr Glu Arg Asp Pro Cys Asp Pro His Lys Gly Leu Phe
 65                  70                  75                  80

Cys Asp Phe Gly Ser Pro Ala Asn Arg Lys Ile Gly Val Cys Thr Ala
                 85                  90                  95

Lys Asp Gly Ala Pro Cys Val Phe Gly Gly Ser Val Tyr Arg Ser Gly
             100                 105                 110
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Glu Ser Phe Gln Ser Ser Cys Lys Tyr Gln Cys Thr Cys Leu Asp Gly
          115     ●         120         ●   125

Ala Val Gly Cys ●️ Pro Leu Cys Ser Met Asp Val A●️u Pro Ser
          130       135             140

Pro Asp Cys Pro Phe Pro Arg Arg Val Lys Leu Pro Gly Lys Cys Cys
145             150             155             160

Glu Glu Trp Val Cys Asp Glu Pro Lys Asp Arg Thr Ala Val Gly Pro
              165             170             175

Ala Leu Ala Ala Tyr Arg Leu Glu Asp Thr Phe Gly Pro Asp Pro Thr
          180             185             190

Met Met Arg Ala Asn Cys Leu Val Gln Thr Thr Glu Trp Ser Ala Cys
          195             200             205

Ser Lys Thr Cys Gly Met Gly Ile Ser Thr Arg Val Thr Asn Asp Asn
    210             215             220

Thr Phe Cys Arg Leu Glu Lys Gln Ser Arg Leu Cys Met Val Arg Pro
225             230             235             240

Cys Glu Ala Asp Leu Glu Glu Asn Ile Lys Lys Gly Lys Lys Cys Ile
          245             250             255

Arg Thr Pro Lys Ile Ala Lys Pro Val Lys Phe Glu Leu Ser Gly Cys
          260             265             270

Thr Ser Val Lys Thr Tyr Arg Ala Lys Phe Cys Gly Val Cys Thr Asp
          275             280             285

Gly Arg Cys Cys Thr Pro His Arg Thr Thr Thr Leu Pro Val Glu Phe
    290             295             300

Lys Cys Pro Asp Gly Glu Ile Met Lys Lys Asn Met Met Phe Ile Lys
305             310             315             320

Thr Cys Ala Cys His Tyr Asn Cys Pro Gly Asp Asn Asp Ile Phe Glu
              325             330             335

Ser Leu Tyr Tyr Arg Lys Met Tyr Gly Asp Met Ala
          340             345

<210> 5
<211> 2345
<212> DNA
<213> Rattus norvegicus

<220>
<223> rat connective tissue growth factor (CTGF) homolog
      cDNA

<220>
<221> CDS
<222> (225)..(1268)
<223> CTGF homolog

5

<400> 5
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gagcgtccgg acaccaacct ccgccccgag accgagtcca ggctcc█c gcgcccctcg 180
tcgcctctgc accccgctgt gcgtcctcct gccgcgcccc gaccatgctc gcctccgtcg 240
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aaaaa 2345


<210> 6
<211> 347
<212> PRT
<213> Rattus norvegicus

<220>
<223> rat connective tissue growth factor (CTGF) homolog

<400> 6
Met Leu Ala Ser Val Ala Gly Pro Val Ser Leu Ala Leu Val Leu Leu
1               5                   10                  15

Leu Cys Thr Arg Pro Ala Thr Gly Gln Asp Cys Ser Ala Gln Cys Gln
            20                  25                  30

Cys Ala Ala Glu Ala Ala Pro Arg Cys Pro Ala Gly Val Ser Leu Val
        35                  40                  45

6

```
Leu Asp Gly Cys Gly Cys Cys Arg Val Cys Ala Lys Gln Leu Gly Glu
    50              55              60

Leu Cys Thr Glu ●  Asp Pro Cys Asp Pro His Lys G ● eu Phe Cys
65              70              75              80

Asp Phe Gly Ser Pro Ala Asn Arg Lys Ile Gly Val Cys Thr Ala Lys
                85              90              95

Asp Gly Ala Pro Cys Val Phe Gly Gly Ser Val Tyr Arg Ser Gly Glu
            100             105             110

Ser Phe Gln Ser Ser Cys Lys Tyr Gln Cys Thr Cys Leu Asp Gly Ala
        115             120             125

Val Gly Cys Val Pro Leu Cys Ser Met Asp Val Arg Leu Pro Ser Pro
    130             135             140

Asp Cys Pro Phe Pro Arg Arg Val Lys Leu Pro Gly Lys Cys Cys Glu
145             150             155             160

Glu Trp Val Cys Asp Glu Pro Lys Asp Arg Thr Val Val Gly Pro Ala
            165             170             175

Leu Ala Ala Tyr Arg Leu Glu Asp Thr Phe Gly Pro Asp Pro Thr Met
            180             185             190

Met Arg Ala Asn Cys Leu Val Gln Thr Thr Glu Trp Ser Ala Cys Ser
        195             200             205

Lys Thr Cys Gly Met Gly Ile Ser Thr Arg Val Thr Asn Asp Asn Thr
    210             215             220

Phe Cys Arg Leu Glu Lys Gln Ser Arg Leu Cys Met Val Arg Pro Cys
225             230             235             240

Glu Ala Asp Leu Glu Glu Asn Ile Lys Lys Gly Lys Lys Cys Ile Arg
            245             250             255

Thr Pro Lys Ile Ala Lys Pro Val Lys Phe Glu Leu Ser Gly Cys Thr
            260             265             270

Ser Val Lys Thr Tyr Arg Ala Lys Phe Cys Gly Val Cys Thr Asp Gly
        275             280             285

Arg Cys Cys Thr Pro His Arg Thr Thr Thr Leu Pro Val Glu Phe Lys
    290             295             300

Cys Pro Asp Gly Glu Ile Met Lys Lys Asn Met Met Phe Ile Lys Thr
305             310             315             320

Cys Ala Cys His Tyr Asn Cys Pro Gly Asp Asn Asp Ile Phe Glu Ser
            325             330             335

Leu Tyr Tyr Arg Lys Met Tyr Gly Asp Met Ala
        340             345
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<210> 7
<211> 3085
<212> DNA
<213> Homo sapie

<220>
<223> human transforming growth factor-beta (TGFB)
      inducible early growth response (TIEG) cDNA

<220>
<221> CDS
<222> (320)..(1729)
<223> TIEG

<400> 7
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gcaattatat tgttagcgat atttcaagag caatggctcg ttttcttagg atttcaacac 180
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<210> 8
<211> 469
<212> PRT
<213> Homo sapiens

<220>
<223> human transforming growth factor-beta (TGFB)
      inducible early growth response (TIEG)

<400> 8
Met Glu Glu Arg Met Glu Met Ile Ser Glu Arg Pro Lys Glu Ser Met
1               5                   10                  15

Tyr Ser Trp Asn Lys Thr Ala Glu Lys Ser Asp Phe Glu Ala Val Glu
            20                  25                  30

Ala Leu Met Ser Met Ser Cys Ser Trp Lys Ser Asp Phe Lys Lys Tyr
        35                  40                  45

Val Glu Asn Arg Pro Val Thr Pro Val Ser Asp Leu Ser Glu Glu Glu
    50                  55                  60

Asn Leu Leu Pro Gly Thr Pro Asp Phe His Thr Ile Pro Ala Phe Cys
65                  70                  75                  80

Leu Thr Pro Pro Tyr Ser Pro Ser Asp Phe Glu Pro Ser Gln Val Ser
                85                  90                  95

Asn Leu Met Ala Pro Ala Pro Ser Thr Val His Phe Lys Ser Leu Ser
            100                 105                 110

Asp Thr Ala Lys Pro His Ile Ala Ala Pro Phe Lys Glu Glu Glu Lys
        115                 120                 125

Ser Pro Val Ser Ala Pro Lys Leu Pro Lys Ala Gln Ala Thr Ser Val
    130                 135                 140

Ile Arg His Thr Ala Asp Ala Gln Leu Cys Asn His Gln Thr Cys Pro
145                 150                 155                 160

Met Lys Ala Ala Ser Ile Leu Asn Tyr Gln Asn Asn Ser Phe Arg Arg
                165                 170                 175

Arg Thr His Leu Asn Val Glu Ala Ala Arg Lys Asn Ile Pro Cys Ala
            180                 185                 190

Ala Val Ser Pro Asn Arg Ser Lys Cys Glu Arg Asn Thr Val Ala Asp
        195                 200                 205

Val Asp Glu Lys Ala Ser Ala Ala Leu Tyr Asp Phe Ser Val Pro Ser
    210                 215                 220

Ser Glu Thr Val Ile Cys Arg Ser Gln Pro Ala Pro Val Ser Pro Gln
225             230             235             240

Gln Lys Ser Val ● Val Ser Pro Pro Ala Val Ser A● ly Gly Val
                245             250             255

Pro Pro Met Pro Val Ile Cys Gln Met Val Pro Leu Pro Ala Asn Asn
        260             265             270

Pro Val Val Thr Thr Val Val Pro Ser Thr Pro Pro Ser Gln Pro Pro
        275             280             285

Ala Val Cys Pro Pro Val Val Phe Met Gly Thr Gln Val Pro Lys Gly
    290             295             300

Ala Val Met Phe Val Val Pro Gln Pro Val Val Gln Ser Ser Lys Pro
305             310             315             320

Pro Val Val Ser Pro Asn Gly Thr Arg Leu Ser Pro Ile Ala Pro Ala
            325             330             335

Pro Gly Phe Ser Pro Ser Ala Ala Lys Val Thr Pro Gln Ile Asp Ser
            340             345             350

Ser Arg Ile Arg Ser His Ile Cys Ser His Pro Gly Cys Gly Lys Thr
        355             360             365

Tyr Phe Lys Ser Ser His Leu Lys Ala His Thr Arg Thr His Thr Gly
    370             375             380

Glu Lys Pro Phe Ser Cys Ser Trp Lys Gly Cys Glu Arg Arg Phe Ala
385             390             395             400

Arg Ser Asp Glu Leu Ser Arg His Arg Arg Thr His Thr Gly Glu Lys
            405             410             415

Lys Phe Ala Cys Pro Met Cys Asp Arg Arg Phe Met Arg Ser Asp His
        420             425             430

Leu Thr Lys His Ala Arg Arg His Leu Ser Ala Lys Lys Leu Pro Asn
    435             440             445

Trp Gln Met Glu Val Ser Lys Leu Asn Asp Ile Ala Leu Pro Pro Thr
    450             455             460

Pro Ala Pro Thr Gln
465


<210> 9
<211> 3045
<212> DNA
<213> Mus musculus

<220>
<223> mouse transforming growth factor-beta (TGFB)
      inducible early growth response (TIEG) homolog
      cDNA

10

<400> 9
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cccaagggtc aaatctgact gcatcagcgc catctactgg ccacttcaaa tctttctccg 480
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<210> 10
<211> 479
<212> PRT
<213> Mus muscul

<220>
<223> mouse transforming growth factor-beta (TGFB)
       inducible early growth response (TIEG) homolog

<400> 10
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 1               5                   10                  15

Glu Leu Ile Ser Glu Lys Pro Arg Glu Gly Met His Pro Trp Asp Lys
            20                  25                  30

Ala Glu Gln Ser Asp Phe Glu Ala Val Glu Ala Leu Met Ser Met Ser
            35                  40                  45

Cys Asp Trp Lys Ser His Phe Lys Lys Tyr Leu Glu Asn Arg Pro Val
    50                  55                  60

Thr Pro Val Ser Asp Thr Ser Glu Asp Asp Ser Leu Leu Pro Gly Thr
65                  70                  75                  80

Pro Asp Leu Gln Thr Val Pro Ala Phe Cys Leu Thr Pro Pro Tyr Ser
                85                  90                  95

Pro Ser Asp Phe Glu Pro Ser Gln Gly Ser Asn Leu Thr Ala Ser Ala
                100                 105                 110

Pro Ser Thr Gly His Phe Lys Ser Phe Ser Asp Ala Ala Lys Pro Pro
            115                 120                 125

Gly Ala Thr Pro Phe Lys Glu Glu Glu Lys Asn Pro Leu Ala Ala Pro
    130                 135                 140

Pro Leu Pro Lys Ala Gln Ala Thr Ser Val Ile Arg His Thr Ala Asp
145                 150                 155                 160

Ala Gln Leu Cys Asn His Gln Ser Cys Pro Val Lys Ala Ala Ser Ile
                165                 170                 175

Leu Asn Tyr Gln Asp Asn Ser Phe Arg Arg Arg Thr His Gly Asn Val
            180                 185                 190

Glu Ala Thr Arg Lys Asn Ile Pro Cys Ala Ala Val Ser Pro Asn Arg
            195                 200                 205

Ser Lys Pro Glu Pro Ser Thr Val Ser Asp Gly Asp Glu Lys Ala Gly
    210                 215                 220

Ala Ala Leu Tyr Asp Phe Ala Val Pro Ser Ser Glu Thr Val Ile Cys
225                 230                 235                 240

Arg Ser Gln Pro Ala Pro Ser Ser Pro Val Gln Lys Ser Val Leu Val
                245                 250                 255

Ser Ser Pro Thr Val Ser Thr Gly Gly Val Pro Pro Leu Pro Val Ile
                260                 265                 270
```

12

```
Cys Gln Met Val Pro Leu Pro Ala Asn Asn Ser Leu Val Ser Thr Val
        275              280                   285
Val Pro Ser Thr Pro Pro Ser Gln Pro Pro Ala Val Cys Ser Pro Val
        290              295                   300
Leu Phe Met Gly Thr Gln Val Pro Glu Gly Thr Val Val Phe Val Val
305              310              315                   320
Pro Gln Pro Val Val Gln Ser Pro Arg Pro Pro Val Val Ser Pro Ser
             325              330                   335
Gly Thr Arg Leu Ser Pro Ile Ala Pro Ala Pro Gly Phe Ser Pro Ser
             340              345                   350
Ala Ala Arg Val Thr Pro Gln Ile Asp Ser Ser Arg Val Arg Ser His
        355              360                   365
Ile Cys Ser His Pro Gly Cys Gly Lys Thr Tyr Phe Lys Ser Ser His
    370              375                   380
Leu Lys Ala His Val Arg Thr His Thr Gly Glu Lys Pro Phe Ser Cys
385              390              395                   400
Ser Trp Lys Gly Cys Glu Arg Arg Phe Ala Arg Ser Asp Glu Leu Ser
             405              410                   415
Arg His Arg Arg Thr His Thr Gly Glu Lys Lys Phe Ala Cys Pro Met
        420              425                   430
Cys Asp Arg Arg Phe Met Arg Ser Asp His Leu Thr Lys His Ala Arg
        435              440                   445
Arg His Leu Ser Ala Lys Lys Leu Pro Asn Trp Gln Met Glu Val Ser
    450              455                   460
Lys Leu Asn Asp Ile Ala Leu Pro Pro Thr Pro Ala Ser Ala Gln
465              470                   475


<210> 11
<211> 3115
<212> DNA
<213> Rattus norvegicus

<220>
<223> rat transforming growth factor-beta (TGFB)
      inducible early growth response (TIEG) homolog
      cDNA

<220>
<221> CDS
<222> (316)..(1758)
<223> TIEG homolog

<400> 11
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ccgctgattg gtcgatacca cgccccgcct tccaccccgc ccgcggcgcg gggcaggcag  180
agcggctacc tgcatgggga gggggggcag acagcgcgga gcgcggtggc gtcgacgtct  240
agtgtctcag tgctccgtct gtggctaggc agccagcagc caagcagctc gcgacctacg  300
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13

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gccaggcagc caaccatgct taacttcggc gcttctctcc agcaagcttc ggaggggaaa  360
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agtgactttg aagc●ga agcgctcatg tccatgagct gcgac●a gtctcacttc  480
aagaaatacc ttgaaаacag gcctgtcaca ccagtgtctg atacc●a ggaagacagt  540
ttgcttccag ggacgcctga ccttcagacc gtcccagcat tctgtttgac tccaccttac  600
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ggtcacttca gatcgctctc cgatgctgcc aagcctccca gcatcgctcc ttttaaagag  720
gaggaaaaga gcccattagc tgcccctccc ctccctaagg ctcaagccac cagtgtcatc  780
cgtcacacag ctgatgccca actctgtaac caccagtcct gccctgtgaa agcagctagc  840
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gcggcgaatg gcgctgagaa ggccggcact gcgccgtatg actttgctgt gccttcctca  1020
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<210> 12
<211> 480
<212> PRT
<213> Rattus norvegicus

<220>
<223> rat transforming growth factor-beta (TGFB)
      inducible early growth response (TIEG) homolog

<400> 12
Met Leu Asn Phe Gly Ala Ser Leu Gln Gln Ala Ser Glu Gly Lys Met
 1               5                   10                  15


14

```
Glu Leu Ile Ser Glu Lys Ser Lys Glu Gly Ala His Pro Trp Asp Lys
              20          25                  30

Ala Glu Gln Ser Asp Phe Glu Ala Val Glu Ala Leu Met Ser Met Ser
         35          40                  45

Cys Asp Trp Lys Ser His Phe Lys Lys Tyr Leu Glu Asn Arg Pro Val
     50              55              60

Thr Pro Val Ser Asp Thr Ser Glu Glu Asp Ser Leu Leu Pro Gly Thr
 65              70              75                      80

Pro Asp Leu Gln Thr Val Pro Ala Phe Cys Leu Thr Pro Pro Tyr Ser
             85              90                      95

Pro Ser Asp Phe Glu Pro Ser Gln Gly Ser Asn Leu Thr Ala Pro Ala
            100             105             110

Pro Pro Thr Gly His Phe Arg Ser Leu Ser Asp Ala Ala Lys Pro Pro
            115             120             125

Ser Ile Ala Pro Phe Lys Glu Glu Glu Lys Ser Pro Leu Ala Ala Pro
    130             135             140

Pro Leu Pro Lys Ala Gln Ala Thr Ser Val Ile Arg His Thr Ala Asp
145             150             155             160

Ala Gln Leu Cys Asn His Gln Ser Cys Pro Val Lys Ala Ala Ser Ile
            165             170             175

Leu Asn Tyr Gln Asp Asn Ser Phe Arg Arg Arg Thr His Ile Asn Val
        180             185             190

Glu Ala Thr Arg Lys Asn Ile Pro Cys Ala Ala Val Ser Pro Asn Arg
        195             200             205

Pro Lys Pro Glu Pro Ser Thr Ala Ala Asn Gly Ala Glu Lys Ala Gly
    210             215             220

Thr Ala Pro Tyr Asp Phe Ala Val Pro Ser Ser Glu Thr Val Ile Cys
225             230             235             240

Arg Ser Ser Gln Pro Ala Pro Thr Ser Pro Val Gln Lys Ser Val Leu
            245             250             255

Met Ser Ser Pro Thr Val Ser Thr Gly Gly Val Pro Pro Leu Pro Val
            260             265             270

Ile Cys Gln Met Val Pro Leu Pro Ala Asn Asn Ser Leu Val Thr Thr
        275             280             285

Val Val Pro Ser Ser Pro Pro Ser Gln Pro Pro Ala Val Cys Ser Pro
    290             295             300

Val Leu Phe Met Gly Thr Gln Val Pro Lys Gly Thr Val Met Phe Val
305             310             315             320

Val Pro Gln Pro Val Val Gln Ser Pro Lys Pro Pro Val Val Ser Pro
            325             330             335
```

15

```
Asn Gly Thr Arg Leu Ser Pro Ile Ala Pro Ala Pro Gly Phe Ser Pro
            340                     345             350

Ser Ala Ala Arg Val Thr Pro Gln Ile Asp Ser Ser Ala Val Arg Ser
            355             360                 365

His Ile Cys Ser His Pro Gly Cys Gly Lys Thr Tyr Phe Lys Ser Ser
    370             375                 380

His Leu Lys Ala His Val Arg Thr His Thr Gly Glu Lys Pro Phe Ser
385                 390                 395                 400

Cys Ser Trp Lys Gly Cys Glu Arg Arg Phe Ala Arg Ser Asp Glu Leu
                405             410             415

Ser Arg His Arg Arg Thr His Thr Gly Glu Lys Lys Phe Ala Cys Pro
            420             425             430

Met Cys Asp Arg Arg Phe Met Arg Ser Asp His Leu Thr Lys His Ala
            435             440             445

Arg Arg His Leu Ser Ala Lys Lys Leu Pro Asn Trp Gln Met Glu Val
    450             455             460

Ser Lys Leu Asn Asp Ile Ala Leu Pro Pro Ala Thr Ala Ser Ala Gln
465             470             475             480


<210> 13
<211> 2872
<212> DNA
<213> Homo sapiens

<220>
<223> human transforming growth factor-beta (TGFB)
      inducible early growth response (TIEG) splice
      variant cDNA

<220>
<221> CDS
<222> (87)..(1529)
<223> TIEG splice variant

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cggaggaaag aatggaaatg atttctgaaa ggccaaaaga gagtatgtat tcctggaaca 180
aaactgcaga gaaaagtgat tttgaagctg tagaagcact tatgtcaatg agctgcagtt 240
ggaagtctga ttttaagaaa tacgttgaaa acagacctgt tacaccagta tctgatttgt 300
cagaggaaga gaatctgctt ccgggaacac tgattttca tacaatccca gcattttgtt 360
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<210> 14
<211> 480
<212> PRT
<213> Homo sapiens

<220>
<223> human transforming growth factor-beta (TGFB)
      inducible early growth response (TIEG) splice
      variant

<400> 14

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Met Leu Asn Phe Gly Ala Ser Leu Gln Gln Thr Ala Glu Glu Arg Met
 1               5                  10                  15

Glu Met Ile Ser Glu Arg Pro Lys Glu Ser Met Tyr Ser Trp Asn Lys
            20                  25                  30

Thr Ala Glu Lys Ser Asp Phe Glu Ala Val Glu Ala Leu Met Ser Met
        35                  40                  45

Ser Cys Ser Trp Lys Ser Asp Phe Lys Lys Tyr Val Glu Asn Arg Pro
    50                  55                  60

Val Thr Pro Val Ser Asp Leu Ser Glu Glu Glu Asn Leu Leu Pro Gly
65                  70                  75                  80

Thr Pro Asp Phe His Thr Ile Pro Ala Phe Cys Leu Thr Pro Pro Tyr
                85                  90                  95
```

Ser Pro Ser Asp Phe Glu Pro Ser Gln Val Ser Asn Leu Met Ala Pro
        100                      105                  110

Ala Pro Ser Thr █ His Phe Lys Ser Leu Ser Asp T█la Lys Pro
        115                120                125

His Ile Ala Ala Pro Phe Lys Glu Glu Glu Lys Ser Pro Val Ser Ala
      130              135              140

Pro Lys Leu Pro Lys Ala Gln Ala Thr Ser Val Ile Arg His Thr Ala
145                150              155              160

Asp Ala Gln Leu Cys Asn His Gln Thr Cys Pro Met Lys Ala Ala Ser
                165              170            175

Ile Leu Asn Tyr Gln Asn Asn Ser Phe Arg Arg Arg Thr His Leu Asn
          180              185              190

Val Glu Ala Ala Arg Lys Asn Ile Pro Cys Ala Ala Val Ser Pro Asn
          195              200            205

Arg Ser Lys Cys Glu Arg Asn Thr Val Ala Asp Val Asp Glu Lys Ala
     210               215              220

Ser Ala Ala Leu Tyr Asp Phe Ser Val Pro Ser Ser Glu Thr Val Ile
225              230              235            240

Cys Arg Ser Gln Pro Ala Pro Val Ser Pro Gln Gln Lys Ser Val Leu
          245              250            255

Val Ser Pro Pro Ala Val Ser Ala Gly Gly Val Pro Pro Met Pro Val
          260              265            270

Ile Cys Gln Met Val Pro Leu Pro Ala Asn Asn Pro Val Val Thr Thr
          275              280            285

Val Val Pro Ser Thr Pro Pro Ser Gln Pro Pro Ala Val Cys Pro Pro
     290               295              300

Val Val Phe Met Gly Thr Gln Val Pro Lys Gly Ala Val Met Phe Val
305              310              315            320

Val Pro Gln Pro Val Val Gln Ser Ser Lys Pro Pro Val Val Ser Pro
          325              330            335

Asn Gly Thr Arg Leu Ser Pro Ile Ala Pro Ala Pro Gly Phe Ser Pro
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Ser Ala Ala Lys Val Thr Pro Gln Ile Asp Ser Ser Arg Ile Arg Ser
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His Ile Cys Ser His Pro Gly Cys Gly Lys Thr Tyr Phe Lys Ser Ser
         370             375            380

His Leu Lys Ala His Thr Arg Thr His Thr Gly Glu Lys Pro Phe Ser
385              390              395            400

Cys Ser Trp Lys Gly Cys Glu Arg Arg Phe Ala Arg Ser Asp Glu Leu
          405              410            415

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    Ser Arg His Arg Arg Thr His Thr Gly Glu Lys Lys Phe Ala Cys Pro
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            435         .   .   440             445

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    Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
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    Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
    65                  70                  75                  80

    Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
                    85                  90                  95
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145                 150                 155                 160

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            165                 170                 175

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
            180                 185                 190

Gly Gly Gly Gly Gly Gly Gly Gly
            195                 200

# PATENT COOPERATION TREATY

NAN Rec'd PCT/PTO 30 NOV 2004

10/516635

From the INTERNATIONAL BUREAU

⬤ T

## NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

HINSCH, Matthew, E.
Townsend and Townsend and Crew LLP
Two Embarcadero Center
Eighth Floor
San Francisco, CA 94111
United States of America

| | |
|---|---|
| **Date of mailing (day/month/year)** 16 July 2003 (16.07.03) | |
| **Applicant's or agent's file reference** 16325-139PC  016325-013900PC | **IMPORTANT NOTIFICATION** |
| **International application No.** PCT/US03/17725 | **International filing date (day/month/year)** 04 June 2003 (04.06.03) |
| **International publication date (day/month/year)** Not yet published | **Priority date (day/month/year)** 04 June 2002 (04.06.02) |
| **Applicant** METABOLEX, INC. et al | |

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An asterisk(*) appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed to Rule 17.1(c)** which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The letters **"NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed to Rule 17.1(c)** which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

| Priority date | Priority application No. | Country or regional Office or PCT receiving Office | Date of receipt of priority document |
|---|---|---|---|
| 04 June 2002 (04.06.02) | 60/386,085 | US | 08 July 2003 (08.07.03) |
| 05 June 2002 (05.06.02) | 60/386,331 | US | 08 July 2003 (08.07.03) |

*Priority Document 10-4-03*

**UNDOCKETED BY** _CW_

| | |
|---|---|
| **The International Bureau of WIPO** 34, chemin des Colombettes 1211 Geneva 20, Switzerland | **Authorized officer** Luis DA COSTA |
| Facsimile No. (41-22) 740-1435 | Telephone No. (41-22) 338.8221 |

Form PCT/IB/304 (July 1998)

005751446

# PCT

NOTICE INFORMING THE APPLICANT OF THE
COMMUNICATION OF THE INTERNATIONAL
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

*MEH*

HINSCH, Matthew, E.
Townsend and Townsend and Crew LLP
Two Embarcadero Center
Eighth Floor
San Francisco, CA 94111
ETATS-UNIS D'AMERIQUE

| | |
|---|---|
| Date of mailing *(day/month/year)*<br>11 December 2003 (11.12.03) | |

| Applicant's or agent's file reference<br>16325-139PC  *016325-013500PC* | **IMPORTANT NOTICE** |

| International application No.<br>PCT/US03/17725 | International filing date *(day/month/year)*<br>04 June 2003 (04.06.03) | Priority date *(day/month/year)*<br>04 June 2002 (04.06.02) |
|---|---|---|

| Applicant<br><br>METABOLEX, INC. et al |
|---|

1. Notice is hereby given that the International Bureau has **communicated**, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this notice:

   AU, AZ, BY, CH, CN, CO, DE, DZ, EP, HU, JP, KG, KP, KR, MD, MK, MZ, RU, TM, US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

   AE, AG, AL, AM, AP, AT, BA, BB, BG, BR, BZ, CA, CR, CU, CZ, DK, DM, EA, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, ID, IL, IN, IS, KE, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MG, MN, MW, MX, NI, NO, NZ, OA, OM, PH, PL, PT, RO, SC, SD, SE, SG, SK, SL, TJ, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-*bis*)).

3. Enclosed with this notice is a copy of the international application as published by the International Bureau on 11 December 2003 (11.12.03) under No. WO 03/102161

4. **TIME LIMITS for filing a demand for international preliminary examination and for entry into the national phase**

   The applicable time limit for entering the national phase will, **subject to what is said in the following paragraph, be 30 MONTHS** from the priority date, not only in respect of any elected Office if a demand for international preliminary examination is filed before the expiration of **19 months** from the priority date, but also in respect of any designated Office, in the absence of filing of such demand, where Article 22(1) as modified with effect from 1 April 2002 applies in respect of that designated Office. For further details, see *PCT Gazette* No. 44/2001 of 1 November 2001, pages 19926, 19932 and 19934, as well as the *PCT Newsletter*, October and November 2001 and February 2002 issues.     *12-4-04*        *2-4-04*

   In practice, **time limits other than the 30-month time limit** will continue to apply, for various periods of time, in respect of certain designated or elected Offices. For **regular updates on the applicable time limits** (20, 21, 30 or 31 months, or other time limit), Office by Office, refer to the *PCT Gazette*, the *PCT Newsletter* and the *PCT Applicant's Guide*, Volume II, National Chapters, all available from WIPO's Internet site, at http://www.wipo.int/pct/en/index.html.

   For filing a demand for international preliminary examination, see the *PCT Applicant's Guide*, Volume I/A, Chapter IX. Only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination (at present, all PCT Contracting States are bound by Chapter II).

   It is the applicant's **sole responsibility** to monitor all these time limits.

   DOCKETED BY _*CJW*_

| The International Bureau of WIPO<br>34, chemin des Colombettes<br>1211 Geneva 20, Switzerland | Authorized officer<br><br>Judith Zahra |
|---|---|
| Facsimile No.(41-22) 740.14.35 | Telephone No.(41-22) 338.91.11 |

Form PCT/IB/308 (April 2002)

From the INTERNATIONAL BUREAU

# PCT

INFORMATION CO●●●NING ELECTED
OFFICES NOTIFIED OF THEIR ELECTION

(PCT Rule 61.3)

To: 016325-013900PC

HINSCH, M●●●, E.
Townsend and Townsend and Crew LLP
Two Embarcadero Center
Eighth Floor
San Francisco, CA 94111
ETATS-UNIS D'AMERIQUE

| Date of mailing (day/month/year) <br> 26 August 2004 (26.08.2004) | |
|---|---|

| Applicant's or agent's file reference <br> 16325-139PC | IMPORTANT INFORMATION |
|---|---|

| International application No. <br> PCT/US2003/017725 | International filing date (day/month/year) <br> 04 June 2003 (04.06.2003) | Priority date (day/month/year) <br> 04 June 2002 (04.06.2002) |
|---|---|---|

| Applicant | METABOLEX, INC. et al |
|---|---|

1. The applicant is hereby informed that the International Bureau has, according to Article 31(7), notified each of the following Offices of its election:

   **EP:** AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR
   **National:** BG, CA, CN, CZ, DE, JP, KP, KR, MN, NO, PL, RO, RU, SK, US

2. The following Offices have waived the requirement for the notification of their election; the notification will be sent to them by the International Bureau only upon their request:

   **AP:** GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW
   **EA:** AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
   **OA:** BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
   **National:** AE, AG, AL, AM, AT, AU, AZ, BA, BB, BR, BY, BZ, CH, CO, CR, CU, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MW, MX, MZ, NI, NZ, OM, PH, PT, SC, SD, SE, SG, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW

3. The applicant is reminded that he must enter the "national phase" **before the expiration of 30 months from the priority date** before each of the Offices listed above. This must be done by paying the national fee(s) and furnishing, if prescribed, a translation of the international application (Article 39(1) (a)), as well as, where applicable, by furnishing a translation of any annexes of the international preliminary examination report (Article 36(3) (b) and Rule 74.1).

   Some offices have fixed time limits expiring later than the above-mentioned time limit. For detailed information about the applicable time limits and the acts to be performed upon entry into the national phase before a particular Office, see Volume II of the PCT Applicant's Guide.

   The entry into European regional phase is postponed **until 31 months from the priority date** for all States designated for the purposes of obtaining a European patent.

| The International Bureau of WIPO <br> 34, chemin des Colombettes <br> 1211 Geneva 20, Switzerland | Authorized officer <br> **Simin Baharlou** |
|---|---|
| Facsimile No.+41 22 740 14 35 | Facsimile No.+41 22 338 71 30 |

Form PCT/IB/332 (September 1997)

(51) International Patent Classification[7]: A61K 39/395, 38/28, 13/00, C12N 15/11, 15/63, C12Q 1/68

(21) International Application Number:
PCT/US2003/017725

(22) International Filing Date: 4 June 2003 (04.06.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/386,085    4 June 2002 (04.06.2002)    US
60/386,331    5 June 2002 (05.06.2002)    US

(71) Applicant (for all designated States except US): METABOLEX, INC. [US/US]; 3876 Bay Center Place, Hayward, CA 94545 (US).

(72) Inventors; and
(75) Inventors/Applicants (for US only): ALLAN, Bernard [IE/US]; 940 Guerrero Street, San Francisco, CA 94110 (US). GREGOIRE, Francine [BE/US]; 1044 Carol Lane, Lafayette, CA 94549 (US). LAVAN, Brian [GB/US]; 2020 Lawton Street, San Francisco, CA 94122 (US). MOODIE, Shonna [GB/US]; 2091 Golden Gate, San Francisco, CA 94115 (US).

(74) Agents: HINSCH, Matthew, E. et al.; Townsend and Townsend and Crew LLP, Two Embarcadero Center, Eighth Floor, San Francisco, CA 94111 (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Declaration under Rule 4.17:**
— of inventorship (Rule 4.17(iv)) for US only

**Published:**
— with international search report
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
5 August 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS OF DIAGNOSING & TREATING DIABETES AND INSULIN RESISTANCE

(57) Abstract: Methods of Diagnosing & Treating Diabetes and Insulin Resistance ABSTRACT OF THE DISCLOSURE The present invention provides compositions and methods for diagnosing and treating diabetes and insulin resistance. In particular, the invention provides methods of identifying modulators of the polynucleotides or polypeptides of the invention and using those modulators to treat diabetes, as well as methods of diagnosing diabetes by measuring the levels of the polynucleotides or polypeptides of the invention in a patient.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/17725

| A. | CLASSIFICATION OF SUBJECT MATTER |
|---|---|
| IPC(7) | : A61K 39/395, 38/28; 13/00; C12N 15/11, 15/63, C12Q 1/68 |
| US CL | : 530/399, 387.9; 435/69.4, 455, 6, 91.1, 375; 536/23.1, 24.5, 25.3 |

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/399, 387.9; 435/69.4, 455, 6, 91.1, 375; 536/23.1, 24.5, 25.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 6,358,741 B1 (SCHMIDT et al.) 19 March 2002 (19.03.2002), SEQ ID NO: 4 | 1-26 |
| Y | US 5,408,040 A (GROTENDORST et al.) 18 April 1995 (18.04.1995), SEQ ID NO: 2 | 1-26 |
| Y | WO 00/35939 A2 (UNIVERSITY OF MIAMI) 22 June 2000 (22.06.2000), Fig. 2A-B | 1-26 |
| Y | WO 00/13706 A1 (HENRY FORD HOSPITAL AND MEDICAL CENTER) 16 March 2000 (16.03.2000), especially pages 5-9 | 1-26 |
| Y | TWIGG S.M. et al., Advanced Glycosylation End Products Up-Regulate Connective Tissue Growth Factor (Insulin-Like Growth Factor-Binding Protein-Related Protein 2) in Human Fibroblasts: A Potential Mechanism for Expansion of Extracellular Matrix in Diabetes Mellitus, Endocrinology 2001, Vol. 142, No. 5, pages 1760-1769, especially page 1768 | 1-26 |
| Y | RISER B.L. et al., Connective tissue growth factor and its regulation: a new element in diabetic glomerulosclerosis, Ren. Fail., 2001 Vol. 23, No. 3-4, pages 459-470, especially pages 469-470 | 1-26 |

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
|---|---|---|---|
| "A" | document defining the general state of the art which is not considered to be of particular relevance | | |
| "E" | earlier application or patent published on or after the international filing date | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | "&" | document member of the same patent family |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 18 February 2004 (18.02.2004) | 25 JUN 2004 |

| Name and mailing address of the ISA/US | Authorized officer |
|---|---|
| Mail Stop PCT, Attn: ISA/US<br>Commissioner for Patents<br>P.O. Box 1450<br>Alexandria, Virginia 22313-1450<br>Facsimile No. (703) 305-3230 | Rachel B. Kapust    Janice Ford<br><br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet) (July 1998)

**Continuation of B. FIELDS SEARCHED Item 3:**
EAST, MEDLINE, CAPLUS, BIOSIS, SCISEARCH
search terms:  SEQ ID NOS: 2, 4, connective tissue growth factor, diabetes, diabetic

*516,635*

(54) Title: METHODS OF DIAGNOSING & TREATING DIABETES AND INSULIN RESISTANCE

(57) Abstract: Methods of Diagnosing & Treating Diabetes and Insulin Resistance ABSTRACT OF THE DISCLOSURE The present invention provides compositions and methods for diagnosing and treating diabetes and insulin resistance. In particular, the invention provides methods of identifying modulators of the polynucleotides or polypeptides of the invention and using those modulators to treat diabetes, as well as methods of diagnosing diabetes by measuring the levels of the polynucleotides or polypeptides of the invention in a patient.

WO 03/102161 A2

# Methods of Diagnosing & Treating Diabetes and Insulin Resistance

## CROSS REFERENCE TO RELATED APPLICATIONS

[01]    The present application claims priority to U.S. Provisional Patent Application No. 60/386,085, filed June 4, 2002 and U.S. Provisional Patent Application No. 60/386,331, June 5, 2002, each of which are incorporated by reference.

## BACKGROUND OF THE INVENTION

[02]    Diabetes mellitus can be divided into two clinical syndromes, Type 1 and Type 2 diabetes mellitus. Type 1, or insulin-dependent diabetes mellitus (IDDM), is a chronic autoimmune disease characterized by the extensive loss of beta cells in the pancreatic Islets of Langerhans, which produce insulin. As these cells are progressively destroyed, the amount of secreted insulin decreases, eventually leading to hyperglycemia (abnormally high level of glucose in the blood) when the amount of secreted insulin drops below the level required for euglycemia (normal blood glucose level). Although the exact trigger for this immune response is not known, patients with IDDM have high levels of antibodies against proteins expressed in pancreatic beta cells. However, not all patients with high levels of these antibodies develop IDDM.

[03]    Type 2 diabetes (also referred to as non-insulin dependent diabetes mellitus (NIDDM)) develops when muscle, fat and liver cells fail to respond normally to insulin. This failure to respond (called insulin resistance) may be due to reduced numbers of insulin receptors on these cells, or a dysfunction of signaling pathways within the cells, or both. The beta cells initially compensate for this insulin resistance by increasing insulin output. Over time, these cells become unable to produce enough insulin to maintain normal glucose levels, indicating progression to Type 2 diabetes.

[04]    Type 2 diabetes is brought on by a combination of genetic and acquired risk factors - including a high-fat diet, lack of exercise, and aging. Worldwide, Type 2 diabetes has become an epidemic, driven by increases in obesity and a sedentary lifestyle, widespread adoption of western dietary habits, and the general aging of the population in many countries. In 1985, an estimated 30 million people worldwide had diabetes -- by 2000, this figure had increased 5-fold, to an estimated 154 million people. The

number of people with diabetes is expected to double between now and 2025, to about 300 million.

[05]      Type 2 diabetes is a complex disease characterized by defects in glucose and lipid metabolism.  Typically there are perturbations in many metabolic parameters including increases in fasting plasma glucose levels, free fatty acid levels and triglyceride levels, as well as a decrease in the ratio of HDL/LDL.  As discussed above, one of the principal underlying causes of diabetes is thought to be an increase in insulin resistance in peripheral tissues, principally muscle and fat.

[06]      Therapies aimed at reducing peripheral insulin resistance are available.  The most relevant to this invention are drugs of the thiazolidinedione (TZD) class namely troglitazone, pioglitazone, and rosiglitazone.  In the US these have been marketed under the names Rezulin™, Avandia™ and Actos™, respectively.  The principal effect of these drugs is to improve glucose homeostasis.  Notably in diabetics treated with TZDs there are increases in peripheral glucose disposal rates indicative of increased insulin sensitivity in both muscle and fat.

[07]      The molecular target of TZDs is a member of the PPAR family of ligand-activated transcription factors called PPAR gamma.  This transcription factor is highly expressed in adipose tissue with much lower levels being observed in muscle.  Binding of TZDs to PPAR gamma in target cells and tissues such as fat and muscle brings about a change in gene expression.  The link between TZD-altered gene expression in fat and muscle and increased insulin sensitivity is unknown.  The present invention addresses this and other problems.

## BRIEF SUMMARY OF THE INVENTION

[08]      The present invention provides methods for identifying an agent for treating a diabetic or pre-diabetic individual.  In some embodiments, the methods comprise the steps of: (i) contacting an agent to a mixture comprising a polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a nucleic acid encoding SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14; and (ii) selecting an agent that modulates the expression or activity of the polypeptide or that binds to the polypeptide, thereby identifying an agent for treating a diabetic or pre-diabetic individual.  In some embodiments, the methods further comprise selecting an agent that modulates insulin sensitivity.

[09]    In some embodiments, step (ii) comprises selecting an agent that modulates expression of the polypeptide. In some embodiments, step (ii) comprises selecting an agent that modulates the activity of the polypeptide. In some embodiments, step (ii) comprises selecting an agent that specifically binds to the polypeptide. In some embodiments, the polypeptide is expressed in a cell and the cell is contacted with the agent.

[10]    The present invention also provides methods of treating a diabetic or pre-diabetic animal. In some embodiments, the methods comprise administering to the animal a therapeutically effective amount of an agent identified as described above. In some embodiments, the agent is an antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the animal is a human.

[11]    The present invention also provides methods of introducing an expression cassette into a cell. In some emodiments, the methods comprise introducing into the cell an expression cassette comprising a promoter operably linked to a polynucleotide encoding a polypeptide, wherein the polynucleotide hybridizes under stringent conditions to a nucleic acid encoding SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

[12]    In some embodiments, the cell is selected from the group consisting of an adipocyte and a skeletal muscle cell.

[13]    In some embodiments, the methods further comprising introducing the cell into a human. In some embodiments, the human is diabetic. In some embodiments, the human is prediabetic. In some embodiments, the cell is from the human.

[14]    The present invention also provides methods of diagnosing an individual who has Type 2 diabetes or is prediabetic. In some embodiments, the method comprises, detecting in a sample from the individual the level of a polypeptide or the level of a polynucleotide encoding the polypeptide, wherein the polynucleotide hybridizes under stringent conditions to a nucleic acid encoding an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14, wherein a modulated level of the polypeptide or polynucleotide in the sample compared to a level of the polypeptide or polynucleotide in either a lean individual or a previous sample from the individual indicates that the individual is diabetic or prediabetic.

[15]    In some embodiments, the detecting step comprises contacting the sample with an antibody that specifically binds to the polypeptide. .

[16]     In some embodiments, the detecting step comprises quantifying

mRNA encoding the polypeptide.  In some embodiments, the mRNA is reverse transcribed

and amplified in a polymerase chain reaction.

[17]     In some embodiments, the sample is a blood, urine or tissue sample.

[18]     The present invention also provides isolated nucleic acids that

hybridize under stringent conditions to a polynucleotide encoding a polypeptide having an

amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4,

SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

[19]     In some embodiments, the nucleic acid is selected from the group

consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9,

SEQ ID NO:11 or SEQ ID NO:13

[20]     The present invention also provides an expression cassette comprising

a heterologous promoter operably linked to a polynucleotide that hybridizes under stringent

conditions to a nucleic acid encoding a polypeptide having an amino acid sequence selected

from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8,

SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

[21]     The present invention also provides host cells transfected with a

polynucleotide that hybridizes under stringent conditions to a nucleic acid encoding a

polypeptide having an amino acid sequence selected from the group consisting of SEQ ID

NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ

ID NO:14.  In some embodiments, the host cell is a human cell.  In some embodiments, the

host cell is a bacterium.

[22]     The present invention also provides isolated polypeptides comprising

an amino acid sequence at least 70% identical to SEQ ID NO: SEQ ID NO:2, SEQ ID NO:4,

SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.  In some

embodiments, the polypeptide is SEQ ID NO: SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6,

SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.


## DEFINITIONS

[23]     "Insulin sensitivity" refers to the ability of a cell or tissue to respond to

insulin.  Responses include, e.g., glucose uptake of a cell or tissue in response to insulin

stimulation.  Sensitivity can be determined at an organismal, tissue or cellular level.  For

example, blood or urine glucose levels following a glucose tolerance test are indicative of

insulin sensitivity.  Other methods of measuring insulin sensitivity include, e.g., measuring

glucose uptake (*see, e.g.,* Garcia de Herreros, A., and Birnbaum, J. *J. Biol. Chem.* 264, 19994-19999 (1989); Klip, A., Li, G., and Logan, W.J. *Am. J. Physiol.* 247, E291-296 (1984)), measuring the glucose infusion rate (GINF) into tissue such as the skeletal muscle (*see, e.g.,* Ludvik *et al., J. Clin. Invest.* 100:2354 (1997); Frias *et al., Diabetes Care* 23:64,

5     (2000)) and measuring sensitivity of GLUT4 translocation (e.g., as described herein) in response to insulin.

[24]     "Activity" of a polypeptide of the invention refers to structural, regulatory, or biochemical functions of a polypeptide in its native cell or tissue.  Examples of activity of a polypeptide include both direct activities and indirect activities.  Exemplary

10    direct activities are the result of direct interaction with the polypeptide, , e.g., enzymatic activity, ligand binding, production or depletion of second messengers (*e.g.,* cAMP, cGMP, $IP_3$, DAG, or $Ca^{2+}$), ion flux, phosphorylation levels, transcription levels, and the like. Exemplary indirect activities are observed as a change in phenotype or response in a cell or tissue to a polypeptide's directed activity, e.g., modulating insulin sensitivity of a cell as a

15    result of the interaction of the polypeptide with other cellular or tissue components.

[25]     "Predisposition for diabetes" occurs in a person when the person is at high risk for developing diabetes.  A number of risk factors are known to those of skill in the art and include: genetic factors (e.g., carrying alleles that result in a higher occurrence of diabetes than in the average population or having parents or siblings with diabetes);

20    overweight (e.g., body mass index (BMI) greater or equal to 25 $kg/m^2$); habitual physical inactivity, race/ethnicity (e.g., African-American, Hispanic-American, Native Americans, Asian-Americans, Pacific Islanders); previously identified impaired fasting glucose or impaired glucose tolerance, hypertension (e.g., greater or equal to 140/90 mmHg in adults); HDL cholesterol less than or equal to 35 mg/dl; triglyceride levels greater or equal to 250

25    mg/dl; a history of gestational diabetes or delivery of a baby over nine pounds; and/or polycystic ovary syndrome.  *See, e.g.,* "Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus" and "Screening for Diabetes" *Diabetes Care* 25(1): S5-S24 (2002).

[26]     A "lean individual," when used to compare with a sample from a

30    patient, refers to an adult with a fasting blood glucose level less than 110 mg/dl or a 2 hour PG reading of 140 mg/dl.  "Fasting"refers to no caloric intake for at least 8 hours.  A "2 hour PG" refers to the level of blood glucose after challenging a patient to a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.  The overall test is generally referred to as an oral glucose tolerance test (OGTT).  *See, e.g., Diabetes Care,*

Supplement 2002, American Diabetes Association: Clinical Practice Recommendations 2002. The level of a polypeptide in a lean individual can be a reading from a single individual, but is typically a statistically relevant average from a group of lean individuals. The level of a polypeptide in a lean individual can be represented by a value, for example in a computer program.

[27]    A "pre-diabetic individual," when used to compare with a sample from a patient, refers to an adult with a fasting blood glucose level greater than 110 mg/dl but less than 126 mg/dl or a 2 hour PG reading of greater than 140 mg/dl but less than 200mg/dl. A "diabetic individual," when used to compare with a sample from a patient, refers to an adult with a fasting blood glucose level greater than 126 mg/dl or a 2 hour PG reading of greater than 200 mg/dl.

[28]    A "diabetes-related nucleic acid" or "diabetes-related polynucleotide" (also referred to as a "nucleic acid of the invention" or a "polynucleotide of the invention") of the invention is a subsequence or full-length polynucleotide sequence of a gene that encodes a polypeptide, whose activity modulates diabetes or insulin sensitivity, or whose presence or absence is indicative of diabetes or altered insulin sensitivity. Exemplary nucleic acids of the invention include those sequences substantially identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13 or encode polypeptides substantially identical to SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.

[29]    An "agonist" refers to an agent that binds to, stimulates, increases, activates, facilitates, enhances activation, sensitizes or up regulates the activity or expression of a polypeptide of the invention.

[30]    An "antagonist" refers to an agent that binds to, partially or totally blocks stimulation, decreases, prevents, delays activation, inactivates, desensitizes, or down regulates the activity or expression of a polypeptide of the invention.

[31]    "Antibody" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[32]     An exemplary immunoglobulin (antibody) structural unit comprises a
tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair
having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus
of each chain defines a variable region of about 100 to 110 or more amino acids primarily
responsible for antigen recognition. The terms variable light chain ($V_L$) and variable heavy
chain ($V_H$) refer to these light and heavy chains respectively.

[33]     Antibodies exist, *e.g.*, as intact immunoglobulins or as a number of
well-characterized fragments produced by digestion with various peptidases. Thus, for
example, pepsin digests an antibody below the disulfide linkages in the hinge region to
produce F(ab)'$_2$, a dimer of Fab which itself is a light chain joined to $V_H$-$C_H1$ by a disulfide
bond. The F(ab)'$_2$ may be reduced under mild conditions to break the disulfide linkage in the
hinge region, thereby converting the F(ab)'$_2$ dimer into an Fab' monomer. The Fab' monomer
is essentially an Fab with part of the hinge region (*see*, Paul (Ed.) *Fundamental Immunology*,
Third Edition, Raven Press, NY (1993)). While various antibody fragments are defined in
terms of the digestion of an intact antibody, one of skill will appreciate that such fragments
may be synthesized *de novo* either chemically or by utilizing recombinant DNA
methodology. Thus, the term antibody, as used herein, also includes antibody fragments
either produced by the modification of whole antibodies or those synthesized *de novo* using
recombinant DNA methodologies (*e.g.*, single chain Fv).

[34]     The terms "peptidomimetic" and "mimetic" refer to a synthetic
chemical compound that has substantially the same structural and functional characteristics of
the antagonists or agonists of the invention. Peptide analogs are commonly used in the
pharmaceutical industry as non-peptide drugs with properties analogous to those of the
template peptide. These types of non-peptide compound are termed "peptide mimetics" or
"peptidomimetics" (Fauchere, J. *Adv. Drug Res.* 15:29 (1986); Veber and Freidinger *TINS* p.
392 (1985); and Evans *et al. J. Med. Chem.* 30:1229 (1987), which are incorporated herein by
reference). Peptide mimetics that are structurally similar to therapeutically useful peptides
may be used to produce an equivalent or enhanced therapeutic or prophylactic effect.
Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a
polypeptide that has a biological or pharmacological activity), such as apolypeptide
exemplified in this application, but have one or more peptide linkages optionally replaced by
a linkage selected from the group consisting of, e.g., -CH2NH-, -CH2S-, -CH2-CH2-, -
CH=CH- (cis and trans), -COCH2-, -CH(OH)CH2-, and -CH2SO-. The mimetic can be
either entirely composed of synthetic, non-natural analogues of amino acids, or, is a chimeric

molecule of partly natural peptide amino acids and partly non-natural analogs of amino acids.
The mimetic can also incorporate any amount of natural amino acid conservative
substitutions as long as such substitutions also do not substantially alter the mimetic's
structure and/or activity. For example, a mimetic composition is within the scope of the
invention if it is capable of carrying out the binding or other activities of an agonist or
antagonist of a polypeptide of the invention.

[35]    The term "gene" means the segment of DNA involved in producing a
polypeptide chain; it includes regions preceding and following the coding region (leader and
trailer) as well as intervening sequences (introns) between individual coding segments
(exons).

[36]    The term "isolated," when applied to a nucleic acid or protein, denotes
that the nucleic acid or protein is essentially free of other cellular components with which it is
associated in the natural state. It is preferably in a homogeneous state although it can be in
either a dry or aqueous solution. Purity and homogeneity are typically determined using
analytical chemistry techniques such as polyacrylamide gel electrophoresis or high
performance liquid chromatography. A protein that is the predominant species present in a
preparation is substantially purified. In particular, an isolated gene is separated from open
reading frames that flank the gene and encode a protein other than the gene of interest. The
term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an
electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least 85% pure,
more preferably at least 95% pure, and most preferably at least 99% pure.

[37]    The term "nucleic acid" or "polynucleotide" refers to
deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-
stranded form. Unless specifically limited, the term encompasses nucleic acids containing
known analogues of natural nucleotides that have similar binding properties as the reference
nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides.
Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses
conservatively modified variants thereof (e.g., degenerate codon substitutions) and
complementary sequences as well as the sequence explicitly indicated. Specifically,
degenerate codon substitutions may be achieved by generating sequences in which the third
position of one or more selected (or all) codons is substituted with mixed-base and/or
deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J.
Biol. Chem. 260:2605-2608 (1985); and Cassol et al. (1992); Rossolini et al., Mol. Cell.

*Probes* 8:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

[38]    The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. As used herein, the terms encompass amino acid chains of any length, including full-length proteins (*i.e.*, antigens), wherein the amino acid residues are linked by covalent peptide bonds.

[39]    The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, $\gamma$-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an $\alpha$ carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but which functions in a manner similar to a naturally occurring amino acid.

[40]    Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[41]    "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids that encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the

corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon

5    in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[42]    As to amino acid sequences, one of skill will recognize that individual

10   substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in

15   the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention.

[43]    The following eight groups each contain amino acids that are conservative substitutions for one another:

1)      Alanine (A), Glycine (G);

20   2)      Aspartic acid (D), Glutamic acid (E);

3)      Asparagine (N), Glutamine (Q);

4)      Arginine (R), Lysine (K);

5)      Isoleucine (I), Leucine (L), Methionine (M), Valine (V);

6)      Phenylalanine (F), Tyrosine (Y), Tryptophan (W);

25   7)      Serine (S), Threonine (T); and

8)      Cysteine (C), Methionine (M)

(see, e.g., Creighton, Proteins (1984)).

[44]    "Percentage of sequence identity" is determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the

30   polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (e.g., a polypeptide of the invention), which does not comprise additions or deletions, for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched

positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

[45]    The terms "identical" or percent "identity," in the context of two or

5    more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same sequences are substantially identical if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, or 95% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum

10    correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. The invention provides polypeptides or polynucleotides that are substantially identical to the polypeptides or polynucleotides, respectively, exemplified herein (e.g., SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ

15    ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12 , SEQ ID NO:13 or SEQ ID NO:14). This definition also refers to the complement of a test sequence. Optionally, the identity exists over a region that is at least about 50 nucleotides in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides in length.

[46]    For sequence comparison, typically one sequence acts as a reference

20    sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test

25    sequences relative to the reference sequence, based on the program parameters.

[47]    A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of

30    contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman (1970) *Adv. Appl. Math.* 2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and

Lipman (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computed implementations of

these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics

Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual

alignment and visual inspection (*see, e.g.,* Ausubel *et al., Current Protocols in Molecular*

5    *Biology* (1995 supplement)).

       [48]    Two examples of algorithms that are suitable for determining percent

sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which

are described in Altschul *et al.* (1977) *Nuc. Acids Res.* 25:3389-3402, and Altschul *et al.*

(1990) *J. Mol. Biol.* 215:403-410, respectively.  Software for performing BLAST analyses is

10   publicly available through the National Center for Biotechnology Information

(http://www.ncbi.nlm.nih.gov/).  This algorithm involves first identifying high scoring

sequence pairs (HSPs) by identifying short words of length W in the query sequence, which

either match or satisfy some positive-valued threshold score T when aligned with a word of

the same length in a database sequence.  T is referred to as the neighborhood word score

15   threshold (Altschul *et al., supra*).  These initial neighborhood word hits act as seeds for

initiating searches to find longer HSPs containing them.  The word hits are extended in both

directions along each sequence for as far as the cumulative alignment score can be increased.

Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward

score for a pair of matching residues; always > 0) and N (penalty score for mismatching

20   residues; always < 0).  For amino acid sequences, a scoring matrix is used to calculate the

cumulative score.  Extension of the word hits in each direction are halted when: the

cumulative alignment score falls off by the quantity X from its maximum achieved value; the

cumulative score goes to zero or below, due to the accumulation of one or more negative-

scoring residue alignments; or the end of either sequence is reached.  The BLAST algorithm

25   parameters W, T, and X determine the sensitivity and speed of the alignment.  The BLASTN

program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation

(E) or 10, M=5, N=-4 and a comparison of both strands.  For amino acid sequences, the

BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the

BLOSUM62 scoring matrix (*see* Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA*

30   89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both

strands.

       [49]    The BLAST algorithm also performs a statistical analysis of the

similarity between two sequences (*see, e.g.,* Karlin and Altschul (1993) *Proc. Natl. Acad. Sci.*

*USA* 90:5873-5787).  One measure of similarity provided by the BLAST algorithm is the

13

smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[50] An indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[51] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (e.g., total cellular or library DNA or RNA).

[52] The phrase "stringent hybridization conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes*, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10° C lower than the thermal melting point ($T_m$) for the specific sequence at a defined ionic strength pH. The $T_m$ is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at $T_m$, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to

50 nucleotides) and at least about 60° C for long probes (*e.g.*, greater than 50 nucleotides).
Stringent conditions may also be achieved with the addition of destabilizing agents such as
formamide. For selective or specific hybridization, a positive signal is at least two times
background, optionally 10 times background hybridization. Exemplary stringent

5    hybridization conditions can be as following: 50% formamide, 5X SSC, and 1% SDS,
incubating at 42°C, or 5X SSC, 1% SDS, incubating at 65°C, with wash in 0.2X SSC, and
0.1% SDS at 55°C, 60°C, or 65°C. Such washes can be performed for 5, 15, 30, 60, 120, or
more minutes.

[53]    Nucleic acids that do not hybridize to each other under stringent

10    conditions are still substantially identical if the polypeptides that they encode are
substantially identical. This occurs, for example, when a copy of a nucleic acid is created
using the maximum codon degeneracy permitted by the genetic code. In such cases, the
nucleic acids typically hybridize under moderately stringent hybridization conditions.
Exemplary "moderately stringent hybridization conditions" include a hybridization in a

15    buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. Such
washes can be performed for 5, 15, 30, 60, 120, or more minutes. A positive hybridization is
at least twice background. Those of ordinary skill will readily recognize that alternative
hybridization and wash conditions can be utilized to provide conditions of similar stringency.

[54]    The phrase "a nucleic acid sequence encoding" refers to a nucleic acid

20    which contains sequence information for a structural RNA such as rRNA, a tRNA, or the
primary amino acid sequence of a specific protein or peptide, or a binding site for a trans-
acting regulatory agent. This phrase specifically encompasses degenerate codons (*i.e.*,
different codons which encode a single amino acid) of the native sequence or sequences that
may be introduced to conform with codon preference in a specific host cell.

25    [55]    The term "recombinant" when used with reference, *e.g.*, to a cell, or
nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has
been modified by the introduction of a heterologous nucleic acid or protein or the alteration
of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus,
for example, recombinant cells express genes that are not found within the native

30    (nonrecombinant) form of the cell or express native genes that are otherwise abnormally
expressed, under-expressed or not expressed at all.

[56]    The term "heterologous" when used with reference to portions of a
nucleic acid indicates that the nucleic acid comprises two or more subsequences that are not
found in the same relationship to each other in nature. For instance, the nucleic acid is

typically recombinantly produced, having two or more sequence om unrelated genes
arranged to make a new functional nucleic acid, *e.g.*, a promoter from one source and a
coding region from another source. Similarly, a heterologous protein indicates that the
protein comprises two or more subsequences that are not found in the same relationship to
each other in nature (*e.g.*, a fusion protein).

[57] An "expression vector" is a nucleic acid construct, generated
recombinantly or synthetically, with a series of specified nucleic acid elements that permit
transcription of a particular nucleic acid in a host cell. The expression vector can be part of a
plasmid, virus, or nucleic acid fragment. Typically, the expression vector includes a nucleic
acid to be transcribed operably linked to a promoter.

[58] The phrase "specifically (or selectively) binds to an antibody" or
"specifically (or selectively) immunoreactive with", when referring to a protein or peptide,
refers to a binding reaction which is determinative of the presence of the protein in the
presence of a heterogeneous population of proteins and other biologics. Thus, under
designated immunoassay conditions, the specified antibodies bind to a particular protein and
do not bind in a significant amount to other proteins present in the sample. Specific binding
to an antibody under such conditions may require an antibody that is selected for its
specificity for a particular protein. For example, antibodies raised against a protein having an
amino acid sequence encoded by any of the polynucleotides of the invention can be selected
to obtain antibodies specifically immunoreactive with that protein and not with other
proteins, except for polymorphic variants. A variety of immunoassay formats may be used to
select antibodies specifically immunoreactive with a particular protein. For example, solid-
phase ELISA immunoassays, Western blots, or immunohistochemistry are routinely used to
select monoclonal antibodies specifically immunoreactive with a protein. *See*, Harlow and
Lane *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, NY (1988) for a
description of immunoassay formats and conditions that can be used to determine specific
immunoreactivity. Typically, a specific or selective reaction will be at least twice the
background signal or noise and more typically more than 10 to 100 times background.

[59] "Inhibitors," "activators," and "modulators" of expression or of
activity are used to refer to inhibitory, activating, or modulating molecules, respectively,
identified using *in vitro* and *in vivo* assays for expression or activity. Modulators encompass
*e.g.*, ligands, agonists, antagonists, and their homologs and mimetics. The term "modulator"
includes inhibitors and activators. Inhibitors are agents that, *e.g.*, inhibit expression of a
polypeptide of the invention or bind to, partially or totally block stimulation, decrease,

prevent, delay activation, inactivate, desensitize, or down regulate activity of a polypeptide of the invention, *e.g.*, antagonists. Activators are agents that, *e.g.*, induce or activate the expression of a polypeptide of the invention or bind to, stimulate, increase, open, activate, facilitate, or enhance activation, sensitize or up regulate the activity of a polypeptide

5   of the invention, *e.g.*, agonists. Modulators include naturally occurring and synthetic ligands, antagonists, agonists, small chemical molecules and the like. Such assays for inhibitors and activators include, *e.g.*, applying putative modulator compounds to cells expressing a polypeptide of the invention and then determining the functional effects on a polypeptide of the invention activity, as described above. Samples or assays comprising a polypeptide of the

10  invention that are treated with a potential activator, inhibitor, or modulator are compared to control samples without the inhibitor, activator, or modulator to examine the extent of effect. Control samples (untreated with modulators) are assigned a relative activity value of 100%. Inhibition of a polypeptide of the invention is achieved when the polypeptide activity value relative to the control is about 80%, optionally 50% or 25, 10%, 5% or 1%. Activation of the

15  polypeptide is achieved when the polypeptide activity value relative to the control is 110%, optionally 150%, optionally 200, 300%, 400%, 500%, or 1000-3000% or more higher.


## DETAILED DESCRIPTION OF THE INVENTION

### I.     INTRODUCTION

20             [60]    The present invention demonstrates that, surprisingly, modulated levels of mRNA comprising sequences of the invention occur in muscle from diabetic individuals treated with thiazolidinedione (TZD) compared to levels of the mRNA in the individuals before TZD treatment. Without intending to limit the invention to a particular mechanism of action, it is believed that modulation of the expression or activity of the polypeptides or

25  polynucleotides of the invention is beneficial in treating diabetic, pre-diabetic or obese insulin resistant, non-diabetic patients. Furthermore, modulated levels of the polypeptides of the invention are indicative of insulin resistance. Thus, the detection of a polypeptide of the invention is useful for diagnosis of diabetes and insulin resistance.

               [61]    This invention also provides methods of using polypeptides of the

30  invention and modulators of the polypeptides of the invention to diagnose and treat diabetes, pre-diabetes (including insulin resistant individuals) and related metabolic diseases. The present method also provides methods of identifying modulators of expression or activity of the polypeptides of the invention. Such modulators are useful for treating Type 2 diabetes as well as the pathological aspects of diabetes (e.g., insulin resistance).

II.     GENERAL RECOMBINANT NUCLEIC ACID METHODS FOR USE
        WITH THE INVENTION

[62]    In numerous embodiments of the present invention, nucleic acids

encoding a polypeptide of the present invention will be isolated and cloned using

5   recombinant methods.  Such embodiments are used, *e.g.*, to isolate polynucleotides identical

or substantially identical to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7,

SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:13 for protein expression or during the

generation of variants, derivatives, expression cassettes, or other sequences derived from an

polypeptide or polynucleotide of the invention, to monitor gene expression, for the isolation

10  or detection of sequences in different species, for diagnostic purposes in a patient, *e.g.*, to

detect mutations in a polypeptide or polynucleotide of the invention or to detect expression

levels of nucleic acids or polypeptides.  In some embodiments, the sequences encoding the

polypeptides of the invention are operably linked to a heterologous promoter.  In one

embodiment, the nucleic acids of the invention are from any mammal, including, in

15  particular, *e.g.*, a human, a mouse, a rat, etc.

A.     General Recombinant Nucleic Acid Methods

[63]    This invention relies on routine techniques in the field of recombinant

genetics.  Basic texts disclosing the general methods of use in this invention include

Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (3rd ed. 2001); Kriegler, *Gene*

20  *Transfer and Expression:  A Laboratory Manual* (1990); and *Current Protocols in Molecular*

*Biology* (Ausubel *et al.*, eds., 1994)).

[64]    For nucleic acids, sizes are given in either kilobases (kb) or base pairs

(bp).  These are estimates derived from agarose or acrylamide gel electrophoresis, from

sequenced nucleic acids, or from published DNA sequences.  For proteins, sizes are given in

25  kilodaltons (kDa) or amino acid residue numbers.  Proteins sizes are estimated from gel

electrophoresis, from sequenced proteins, from derived amino acid sequences, or from

published protein sequences.

[65]    Oligonucleotides that are not commercially available can be

chemically synthesized according to the solid phase phosphoramidite triester method first

30  described by Beaucage & Caruthers, *Tetrahedron Letts.* 22:1859-1862 (1981), using an

automated synthesizer, as described in Van Devanter *et. al.*, *Nucleic Acids Res.* 12:6159-6168

(1984).  Purification of oligonucleotides is by either native acrylamide gel electrophoresis or

by anion-exchange HPLC as described in Pearson & Reanier, *J. Chrom.* 255:137-149 (1983).

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[66]   The sequence of the cloned genes and synthetic oligonucleotides can be verified after cloning using, e.g., the chain termination method for sequencing double-stranded templates of Wallace *et al., Gene* 16:21-26 (1981).

**B.     Cloning Methods for the Isolation of Nucleotide Sequences Encoding Desired Proteins**

[67]   In general, the nucleic acids encoding the subject proteins are cloned from DNA sequence libraries that are made to encode cDNA or genomic DNA. The particular sequences can be located by hybridizing with an oligonucleotide probe, the sequence of which can be derived from the sequences disclosed herein, which provide a reference for PCR primers and defines suitable regions for isolating probes pecific for the polypeptides or polynucleotides of the invention. Alternatively, where the sequence is cloned into an expression library, the expressed recombinant protein can be detected immunologically with antisera or purified antibodies made against a polypeptide of interest, including those disclosed herein.

[68]   Methods for making and screening genomic and cDNA libraries are well known to those of skill in the art (*see, e.g.,* Gubler and Hoffman *Gene* 25:263-269 (1983); Benton and Davis *Science,* 196:180-182 (1977); and Sambrook, *supra*).

[69]   Briefly, to make the cDNA library, one should choose a source that is rich in mRNA. The mRNA can then be made into cDNA, ligated into a recombinant vector, and transfected into a recombinant host for propagation, screening and cloning. For a genomic library, the DNA is extracted from a suitable tissue and either mechanically sheared or enzymatically digested to yield fragments of preferably about 5-100 kb. The fragments are then separated by gradient centrifugation from undesired sizes and are constructed in bacteriophage lambda vectors. These vectors and phage are packaged *in vitro,* and the recombinant phages are analyzed by plaque hybridization. Colony hybridization is carried out as generally described in Grunstein *et al., Proc. Natl. Acad. Sci. USA.,* 72:3961-3965 (1975).

[70]   An alternative method combines the use of synthetic oligonucleotide primers with polymerase extension on an mRNA or DNA template. Suitable primers can be designed from specific sequences disclosed herein. This polymerase chain reaction (PCR) method amplifies the nucleic acids encoding the protein of interest directly from mRNA, cDNA, genomic libraries or cDNA libraries. Restriction endonuclease sites can be incorporated into the primers. Polymerase chain reaction or other *in vitro* amplification methods may also be useful, for example, to clone nucleic acids encoding specific proteins

and express said proteins, to synthesize nucleic acids that will be used as probes for detecting the presence of mRNA encoding a polypeptide of the invention in physiological samples, for nucleic acid sequencing, or for other purposes (*see*, U.S. Patent Nos. 4,683,195 and 4,683,202). Genes amplified by a PCR reaction can be purified from agarose gels and cloned into an appropriate vector.

[71]   Appropriate primers and probes for identifying the genes encoding a polypeptide of the invention from mammalian tissues can be derived from the sequences provided herein. For a general overview of PCR, *see*, Innis *et al. PCR Protocols: A Guide to Methods and Applications*, *Academic Press*, San Diego (1990).

[72]   Synthetic oligonucleotides can be used to construct genes. This is done using a series of overlapping oligonucleotides, usually 40-120 bp in length, representing both the sense and anti-sense strands of the gene. These DNA fragments are then annealed, ligated and cloned.

[73]   A polynucleotide encoding a polypeptide of the invention can be cloned using intermediate vectors before transformation into mammalian cells for expression. These intermediate vectors are typically prokaryote vectors or shuttle vectors. The proteins can be expressed in either prokaryotes or eukaryotes, using standard methods well known to those of skill in the art.

**III.    PURIFICATION OF PROTEINS OF THE INVENTION**

[74]   Either naturally occurring or recombinant polypeptides of the invention can be purified for use in functional assays. Naturally occurring polypeptides of the invention can be purified from any source (e.g., tissues of an organism expressing an ortholog). Recombinant polypeptides can be purified from any suitable expression system.

[75]   The polypeptides of the invention may be purified to substantial purity by standard techniques, including selective precipitation with such substances as ammonium sulfate; column chromatography, immunopurification methods, and others (*see, e.g.*, Scopes, *Protein Purification: Principles and Practice* (1982); U.S. Patent No. 4,673,641; Ausubel *et al., supra;* and Sambrook *et al., supra*).

[76]   A number of procedures can be employed when recombinant polypeptides are being purified. For example, proteins having established molecular adhesion properties can be reversibly fused to a polypeptide of the invention. With the appropriate ligand, either protein can be selectively adsorbed to a purification column and

then freed from the column in a relatively pure form. The fused protein may be then removed by enzymatic activity. Finally polypeptides can be purified using immunoaffinity columns.

### A.      Purification of Proteins from Recombinant Bacteria

[77]    When recombinant proteins are expressed by the transformed bacteria in large amounts, typically after promoter induction, although expression can be constitutive, the proteins may form insoluble aggregates. There are several protocols that are suitable for purification of protein inclusion bodies. For example, purification of aggregate proteins (hereinafter referred to as inclusion bodies) typically involves the extraction, separation and/or purification of inclusion bodies by disruption of bacterial cells typically, but not limited to, by incubation in a buffer of about 100-150 µg/ml lysozyme and 0.1% Nonidet P40, a non-ionic detergent. The cell suspension can be ground using a Polytron grinder (Brinkman Instruments, Westbury, NY). Alternatively, the cells can be sonicated on ice. Alternate methods of lysing bacteria are described in Ausubel *et al.* and Sambrook *et al.*, both *supra*, and will be apparent to those of skill in the art.

[78]    The cell suspension is generally centrifuged and the pellet containing the inclusion bodies resuspended in buffer which does not dissolve but washes the inclusion bodies, *e.g.*, 20 mM Tris-HCl (pH 7.2), 1 mM EDTA, 150 mM NaCl and 2% Triton-X 100, a non-ionic detergent. It may be necessary to repeat the wash step to remove as much cellular debris as possible. The remaining pellet of inclusion bodies may be resuspended in an appropriate buffer (*e.g.*, 20 mM sodium phosphate, pH 6.8, 150 mM NaCl). Other appropriate buffers will be apparent to those of skill in the art.

[79]    Following the washing step, the inclusion bodies are solubilized by the addition of a solvent that is both a strong hydrogen acceptor and a strong hydrogen donor (or a combination of solvents each having one of these properties). The proteins that formed the inclusion bodies may then be renatured by dilution or dialysis with a compatible buffer. Suitable solvents include, but are not limited to, urea (from about 4 M to about 8 M), formamide (at least about 80%, volume/volume basis), and guanidine hydrochloride (from about 4 M to about 8 M). Some solvents that are capable of solubilizing aggregate-forming proteins, such as SDS (sodium dodecyl sulfate) and 70% formic acid, are inappropriate for use in this procedure due to the possibility of irreversible denaturation of the proteins, accompanied by a lack of immunogenicity and/or activity. Although guanidine hydrochloride and similar agents are denaturants, this denaturation is not irreversible and renaturation may occur upon removal (by dialysis, for example) or dilution of the denaturant,

allowing re-formation of the immunologically and/or biologically active protein of interest. After solubilization, the protein can be separated from other bacterial proteins by standard separation techniques.

[80]    Alternatively, it is possible to purify proteins from bacteria periplasm. Where the protein is exported into the periplasm of the bacteria, the periplasmic fraction of the bacteria can be isolated by cold osmotic shock in addition to other methods known to those of skill in the art (see, Ausubel et al., supra). To isolate recombinant proteins from the periplasm, the bacterial cells are centrifuged to form a pellet. The pellet is resuspended in a buffer containing 20% sucrose. To lyse the cells, the bacteria are centrifuged and the pellet is resuspended in ice-cold 5 mM MgSO$_4$ and kept in an ice bath for approximately 10 minutes. The cell suspension is centrifuged and the supernatant decanted and saved. The recombinant proteins present in the supernatant can be separated from the host proteins by standard separation techniques well known to those of skill in the art.


**B.      Purification of Proteins from Insect Cells**

[81]    Proteins can also be purified from eukaryotic gene expression systems as described in, e.g., Fernandez and Hoeffler, *Gene Expression Systems* (1999). In some embodiments, baculovirus expression systems are used to isolate proteins of the invention. Recombinant baculoviruses are generally generated by replacing the polyhedrin coding sequence of a baculovirus with a gene to be expressed (e.g., encoding a polypeptide of the invention). Viruses lacking the polyhedrin gene have a unique plaque morphology making them easy to recognize. In some embodiments, a recombinant baculovirus is generated by first cloning a polynucleotide of interest into a transfer vector (e.g., a pUC based vector) such that the polynucleotide is operably linked to a polyhedrin promoter. The transfer vector is transfected with wildtype DNA into an insect cell (e.g., Sf9, Sf21 or BT1-TN-5B1-4 cells), resulting in homologous recombination and replacement of the polyhedrin gene in the wildtype viral DNA with the polynucleotide of interest. Virus can then be generated and plaque purified. Protein expression results upon viral infection of insect cells. Expressed proteins can be harvested from cell supernatant if secreted, or from cell lysates if intracellular. *See, e.g.,* Ausubel *et al.* and Fernandez and Hoeffler, *supra.*

C.      Standard Protein Separation Techniques For Purifying Proteins

### 1. Solubility Fractionation

[82]    Often as an initial step, and if the protein mixture is complex, an initial
salt fractionation can separate many of the unwanted host cell proteins (or proteins derived
from the cell culture media) from the recombinant protein of interest. The preferred salt is
ammonium sulfate. Ammonium sulfate precipitates proteins by effectively reducing the
amount of water in the protein mixture. Proteins then precipitate on the basis of their
solubility. The more hydrophobic a protein is, the more likely it is to precipitate at lower
ammonium sulfate concentrations. A typical protocol is to add saturated ammonium sulfate
to a protein solution so that the resultant ammonium sulfate concentration is between 20-
30%. This will precipitate the most hydrophobic proteins. The precipitate is discarded
(unless the protein of interest is hydrophobic) and ammonium sulfate is added to the
supernatant to a concentration known to precipitate the protein of interest. The precipitate is
then solubilized in buffer and the excess salt removed if necessary, through either dialysis or
diafiltration. Other methods that rely on solubility of proteins, such as cold ethanol
precipitation, are well known to those of skill in the art and can be used to fractionate
complex protein mixtures.

### 2. Size Differential Filtration

[83]    Based on a calculated molecular weight, a protein of greater and lesser
size can be isolated using ultrafiltration through membranes of different pore sizes (for
example, Amicon or Millipore membranes). As a first step, the protein mixture is
ultrafiltered through a membrane with a pore size that has a lower molecular weight cut-off
than the molecular weight of the protein of interest. The retentate of the ultrafiltration is then
ultrafiltered against a membrane with a molecular cut off greater than the molecular weight of
the protein of interest. The recombinant protein will pass through the membrane into the
filtrate. The filtrate can then be chromatographed as described below.

### 3. Column Chromatography

[84]    The proteins of interest can also be separated from other proteins on
the basis of their size, net surface charge, hydrophobicity and affinity for ligands. In
addition, antibodies raised against proteins can be conjugated to column matrices and the
proteins immunopurified. All of these methods are well known in the art.

[85]    Immunoaffinity chromatography using antibodies raised to a variety of
affinity tags such as hemagglutinin (HA), FLAG, Xpress, Myc, hexahistidine (His),
glutathione S transferase (GST) and the like can be used to purify polypeptides. The His tag

will also act as a chelating agent for certain metals (e.g., Ni) and s the metals can also be used to purify His-containing polypeptides. After purification, the tag is optionally removed by specific proteolytic cleavage.

[86]     It will be apparent to one of skill that chromatographic techniques can be performed at any scale and using equipment from many different manufacturers (*e.g.,* Pharmacia Biotech).

## IV.    DETECTION OF POLYNUCLEOTIDES OF THE INVENTION

[87]     Those of skill in the art will recognize that detection of expression of polynucleotides and polypeptides of the invention has many uses. For example, as discussed herein, detection of levels of polynucleotides and polypeptides of the invention in a patient is useful for diagnosing diabetes or a predisposition for at least some of the pathological effects of diabetes. Moreover, detection of gene expression is useful to identify modulators of expression of polynucleotides and polypeptides of the invention.

[88]     A variety of methods of specific DNA and RNA measurement that use nucleic acid hybridization techniques are known to those of skill in the art (*see,* Sambrook, *supra*). Some methods involve an electrophoretic separation (*e.g.,* Southern blot for detecting DNA, and Northern blot for detecting RNA), but measurement of DNA and RNA can also be carried out in the absence of electrophoretic separation (*e.g.,* by dot blot). Southern blot of genomic DNA (*e.g.,* from a human) can be used for screening for restriction fragment length polymorphism (RFLP) to detect the presence of a genetic disorder affecting a polypeptide of the invention.

[89]     The selection of a nucleic acid hybridization format is not critical. A variety of nucleic acid hybridization formats are known to those skilled in the art. For example, common formats include sandwich assays and competition or displacement assays. Hybridization techniques are generally described in Hames and Higgins *Nucleic Acid Hybridization, A Practical Approach,* IRL Press (1985); Gall and Pardue, *Proc. Natl. Acad. Sci. U.S.A.,* 63:378-383 (1969); and John *et al. Nature,* 223:582-587 (1969).

[90]     Detection of a hybridization complex may require the binding of a signal-generating complex to a duplex of target and probe polynucleotides or nucleic acids. Typically, such binding occurs through ligand and anti-ligand interactions as between a ligand-conjugated probe and an anti-ligand conjugated with a signal. The binding of the signal generation complex is also readily amenable to accelerations by exposure to ultrasonic energy.

[91]    The label may also allow indirect detection of the hybridization complex. For example, where the label is a hapten or antigen, the sample can be detected by using antibodies. In these systems, a signal is generated by attaching fluorescent or enzyme molecules to the antibodies or in some cases, by attachment to a radioactive label (*see, e.g.,*

5    Tijssen, "*Practice and Theory of Enzyme Immunoassays,*" *Laboratory Techniques in Biochemistry and Molecular Biology*, Burdon and van Knippenberg Eds., Elsevier (1985), pp. 9-20).

[92]    The probes are typically labeled either directly, as with isotopes, chromophores, lumiphores, chromogens, or indirectly, such as with biotin, to which a

10    streptavidin complex may later bind. Thus, the detectable labels used in the assays of the present invention can be primary labels (where the label comprises an element that is detected directly or that produces a directly detectable element) or secondary labels (where the detected label binds to a primary label, *e.g.*, as is common in immunological labeling). Typically, labeled signal nucleic acids are used to detect hybridization. Complementary

15    nucleic acids or signal nucleic acids may be labeled by any one of several methods typically used to detect the presence of hybridized polynucleotides. The most common method of detection is the use of autoradiography with $^{3}$H, $^{125}$I, $^{35}$S, $^{14}$C, or $^{32}$P-labeled probes or the like.

[93]    Other labels include, *e.g.*, ligands that bind to labeled antibodies,

20    fluorophores, chemiluminescent agents, enzymes, and antibodies that can serve as specific binding pair members for a labeled ligand. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden *Introduction to Immunocytochemistry*, 2nd ed., Springer Verlag, NY (1997); and in Haugland *Handbook of Fluorescent Probes and Research Chemicals*, a combined handbook and catalogue Published by Molecular Probes,

25    Inc. (1996).

[94]    In general, a detector that monitors a particular probe or probe combination is used to detect the detection reagent label. Typical detectors include spectrophotometers, phototubes and photodiodes, microscopes, scintillation counters, cameras, film and the like, as well as combinations thereof. Examples of suitable detectors

30    are widely available from a variety of commercial sources known to persons of skill in the art. Commonly, an optical image of a substrate comprising bound labeling moieties is digitized for subsequent computer analysis.

[95]    The amount of, for example, an RNA is measured by quantitating the amount of label fixed to the solid support by binding of the detection reagent. Typically, the

presence of a modulator during incubation will increase or decrease the amount of label fixed to the solid support relative to a control incubation that does not comprise the modulator, or as compared to a baseline established for a particular reaction type. Means of detecting and quantitating labels are well known to those of skill in the art.

[96] In some embodiments, the target nucleic acid or the probe is immobilized on a solid support. Solid supports suitable for use in the assays of the invention are known to those of skill in the art. As used herein, a solid support is a matrix of material in a substantially fixed arrangement.

[97] A variety of automated solid-phase assay techniques are also appropriate. For instance, very large scale immobilized polymer arrays (VLSIPS™), i.e. Gene Chips or microarrays, available from Affymetrix, Inc. in Santa Clara, CA can be used to detect changes in expression levels of a plurality of genes involved in the same regulatory pathways simultaneously. See, Tijssen, supra., Fodor et al. (1991) Science, 251: 767- 777; Sheldon et al. (1993) Clinical Chemistry 39(4): 718-719, and Kozal et al. (1996) Nature Medicine 2(7): 753-759. Similarly, spotted cDNA arrays (arrays of cDNA sequences bound to nylon, glass or another solid support) can also be used to monitor expression of a plurality of genes.

[98] Typically, the array elements are organized in an ordered fashion so that each element is present at a specified location on the substrate. Because the array elements are at specified locations on the substrate, the hybridization patterns and intensities (which together create a unique expression profile) can be interpreted in terms of expression levels of particular genes and can be correlated with a particular disease or condition or treatment. See, e.g., Schena et al., Science 270: 467-470 (1995)) and (Lockhart et al., Nature Biotech. 14: 1675-1680 (1996)).

[99] Hybridization specificity can be evaluated by comparing the hybridization of specificity-control polynucleotide sequences to specificity-control polynucleotide probes that are added to a sample in a known amount. The specificity-control target polynucleotides may have one or more sequence mismatches compared with the corresponding polynucleotide sequences. In this manner, whether only complementary target polynucleotides are hybridizing to the polynucleotide sequences or whether mismatched hybrid duplexes are forming is determined.

[100] Hybridization reactions can be performed in absolute or differential hybridization formats. In the absolute hybridization format, polynucleotide probes from one sample are hybridized to the sequences in a microarray format and signals detected after

hybridization complex formation correlate to polynucleotide probe levels in a sample. In the differential hybridization format, the differential expression of a set of genes in two biological samples is analyzed. For differential hybridization, polynucleotide probes from both biological samples are prepared and labeled with different labeling moieties. A mixture

5   of the two labeled polynucleotide probes is added to a microarray. The microarray is then examined under conditions in which the emissions from the two different labels are individually detectable. Sequences in the microarray that are hybridized to substantially equal numbers of polynucleotide probes derived from both biological samples give a distinct combined fluorescence (Shalon *et al.* PCT publication WO95/35505). In some embodiments,

10  the labels are fluorescent labels with distinguishable emission spectra, such as Cy3 and Cy5 fluorophores.

[101] After hybridization, the microarray is washed to remove nonhybridized nucleic acids and complex formation between the hybridizable array elements and the polynucleotide probes is detected. Methods for detecting complex formation are well known

15  to those skilled in the art. In some embodiments, the polynucleotide probes are labeled with a fluorescent label and measurement of levels and patterns of fluorescence indicative of complex formation is accomplished by fluorescence microscopy, such as confocal fluorescence microscopy.

[102] In a differential hybridization experiment, polynucleotide probes from

20  two or more different biological samples are labeled with two or more different fluorescent labels with different emission wavelengths. Fluorescent signals are detected separately with different photomultipliers set to detect specific wavelengths. The relative abundances/expression levels of the polynucleotide probes in two or more samples are obtained.

25          [103] Typically, microarray fluorescence intensities can be normalized to take into account variations in hybridization intensities when more than one microarray is used under similar test conditions. In some embodiments, individual polynucleotide probe/target complex hybridization intensities are normalized using the intensities derived from internal normalization controls contained on each microarray.

30          [104] Detection of nucleic acids can also be accomplished, for example, by using a labeled detection moiety that binds specifically to duplex nucleic acids (*e.g.,* an antibody that is specific for RNA-DNA duplexes). One example uses an antibody that recognizes DNA-RNA heteroduplexes in which the antibody is linked to an enzyme (typically by recombinant or covalent chemical bonding). The antibody is detected when the

enzyme reacts with its substrate, producing a detectable product. Butlee *et al.* (1989)

*Analytical Biochemistry* 181:153-162; Bogulavski (1986) *et al. J. Immunol. Methods* 89:123-

130; Prooijen-Knegt (1982) *Exp. Cell Res.* 141:397-407; Rudkin (1976) *Nature* 265:472-473,

Stollar (1970) *PNAS* 65:993-1000; Ballard (1982) *Mol. Immunol.* 19:793-799; Pisetsky and

5    Caster (1982) *Mol. Immunol.* 19:645-650; Viscidi *et al.* (1988) *J. Clin. Microbial.* 41:199-

209; and Kiney *et al.* (1989) *J. Clin. Microbiol.* 27:6-12 describe antibodies to RNA

duplexes, including homo and heteroduplexes. Kits comprising antibodies specific for

DNA:RNA hybrids are available, *e.g.*, from Digene Diagnostics, Inc. (Beltsville, MD).

[105]   In addition to available antibodies, one of skill in the art can easily

10   make antibodies specific for nucleic acid duplexes using existing techniques, or modify those

antibodies that are commercially or publicly available. In addition to the art referenced

above, general methods for producing polyclonal and monoclonal antibodies are known to

those of skill in the art (*see, e.g.*, Paul (ed) *Fundamental Immunology, Third Edition* Raven

Press, Ltd., NY (1993); Coligan *Current Protocols in Immunology* Wiley/Greene, NY

15   (1991); Harlow and Lane *Antibodies: A Laboratory Manual* Cold Spring Harbor Press, NY

(1989); Stites *et al.* (eds.) *Basic and Clinical Immunology* (4th ed.) Lange Medical

Publications, Los Altos, CA, and references cited therein; Goding *Monoclonal Antibodies:*

*Principles and Practice* (2d ed.) Academic Press, New York, NY, (1986); and Kohler and

Milstein *Nature* 256: 495-497 (1975)). Other suitable techniques for antibody preparation

20   include selection of libraries of recombinant antibodies in phage or similar vectors (*see,* Huse

*et al. Science* 246:1275-1281 (1989); and Ward *et al. Nature* 341:544-546 (1989)). Specific

monoclonal and polyclonal antibodies and antisera will usually bind with a $K_D$ of at least

about 0.1 μM, preferably at least about 0.01 μM or better, and most typically and preferably,

0.001 μM or better.

25              [106]   The nucleic acids used in this invention can be either positive or

negative probes. Positive probes bind to their targets and the presence of duplex formation is

evidence of the presence of the target. Negative probes fail to bind to the suspect target and

the absence of duplex formation is evidence of the presence of the target. For example, the

use of a wild type specific nucleic acid probe or PCR primers may serve as a negative probe

30   in an assay sample where only the nucleotide sequence of interest is present.

[107]   The sensitivity of the hybridization assays may be enhanced through

use of a nucleic acid amplification system that multiplies the target nucleic acid being

detected. Examples of such systems include the polymerase chain reaction (PCR) system and

the ligase chain reaction (LCR) system. Other methods recently described in the art are the

nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario) and Q
Beta Replicase systems.  These systems can be used to directly identify mutants where the
PCR or LCR primers are designed to be extended or ligated only when a selected sequence is
present.  Alternatively, the selected sequences can be generally amplified using, for example,

5       nonspecific PCR primers and the amplified target region later probed for a specific sequence
indicative of a mutation.  It is understood that various detection probes, including Taqman
and molecular beacon probes can be used to monitor amplification reaction products, e.g., in
real time.

        [108]   An alternative means for determining the level of expression of the

10      nucleic acids of the present invention is *in situ* hybridization.  *In situ* hybridization assays are
well known and are generally described in Angerer *et al., Methods Enzymol.* 152:649-660
(1987).  In an *in situ* hybridization assay, cells, preferentially human cells from the
cerebellum or the hippocampus, are fixed to a solid support, typically a glass slide.  If DNA is
to be probed, the cells are denatured with heat or alkali.  The cells are then contacted with a

15      hybridization solution at a moderate temperature to permit annealing of specific probes that
are labeled.  The probes are preferably labeled with radioisotopes or fluorescent reporters.

        [109]   Single nucleotide polymorphism (SNP) analysis is also useful for
detecting differences between alleles of the polynucleotides (e.g., genes) of the invention.
SNPs linked to genes encoding polypeptides of the invention are useful, for instance, for

20      diagnosis of diseases (e.g., diabetes) whose occurrence is linked to the gene sequences of the
invention.  For example, if an individual carries at least one SNP linked to a disease-
associated allele of the gene sequences of the invention, the individual is likely predisposed
for one or more of those diseases.  If the individual is homozygous for a disease-linked SNP,
the individual is particularly predisposed for occurrence of that disease (e.g., diabetes).  In

25      some embodiments, the SNP associated with the gene sequences of the invention is located
within 300,000; 200,000; 100,000; 75,000; 50,000; or 10,000 base pairs from the gene
sequence.

        [110]   Various real-time PCR methods including, e.g., Taqman or molecular
beacon-based assays (e.g., U.S. Patent Nos. 5,210,015; 5,487,972; Tyagi *et al., Nature*

30      *Biotechnology* 14:303 (1996); and PCT WO 95/13399 are useful to monitor for the presence
of absence of a SNP.  Additional SNP detection methods include, e.g., DNA sequencing,
sequencing by hybridization, dot blotting, oligonucleotide array (DNA Chip) hybridization
analysis, or are described in, e.g., U.S. Patent No. 6,177,249; Landegren *et al., Genome
Research*, 8:769-776 (1998); Botstein *et al., Am J Human Genetics* 32:314-331 (1980);

Meyers *et al.*, Methods in Enzymology 155:501-527 (1987); Kee _ al., *Trends in Genetics* 7:5 (1991); Myers *et al.*, *Science* 230:1242-1246 (1985); and Kwok *et al.*, *Genomics* 23:138-144 (1994).

## V.     IMMUNOLOGICAL DETECTION OF POLYPEPTIDES OF THE INVENTION

[111]    In addition to the detection of polynucloetides of the invention and gene expression using nucleic acid hybridization technology, one can also use immunoassays to detect polypeptides of the invention. Immunoassays can be used to qualitatively or quantitatively analyze polypeptides of the invention. A general overview of the applicable technology can be found in Harlow & Lane, *Antibodies: A Laboratory Manual* (1988).

### A.     Antibodies to Target Proteins or other immunogens

[112]    Methods for producing polyclonal and monoclonal antibodies that react specifically with a protein of interest or other immunogen are known to those of skill in the art (*see, e.g.,* Coligan, *supra*; and Harlow and Lane, *supra*; Stites *et al.*, *supra* and references cited therein; Goding, *supra*; and Kohler and Milstein *Nature*, 256:495-497 (1975)). Such techniques include antibody preparation by selection of antibodies from libraries of recombinant antibodies in phage or similar vectors (*see*, Huse *et al.*, *supra*; and Ward *et al.*, *supra*). For example, in order to produce antisera for use in an immunoassay, the protein of interest or an antigenic fragment thereof, is isolated as described herein. For example, a recombinant protein is produced in a transformed cell line. An inbred strain of mice or rabbits is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard immunization protocol. Alternatively, a synthetic peptide derived from the sequences disclosed herein is conjugated to a carrier protein and used as an immunogen.

[113]    Polyclonal sera are collected and titered against the immunogen in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of $10^4$ or greater are selected and tested for their crossreactivity against proteins other than the polypeptides of the invention or even other homologous proteins from other organisms, using a competitive binding immunoassay. Specific monoclonal and polyclonal antibodies and antisera will usually bind with a $K_D$ of at least about 0.1 mM, more usually at least about 1 μM, preferably at least about 0.1 μM or better, and most preferably, 0.01 μM or better.

[114]    A number of proteins of the invention comprising immunogens may be used to produce antibodies specifically or selectively reactive with the proteins of interest. Recombinant protein is the preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein may also be used either in pure or impure

5    form. Synthetic peptides made using the protein sequences described herein may also be used as an immunogen for the production of antibodies to the protein. Recombinant protein can be expressed in eukaryotic or prokaryotic cells and purified as generally described *supra*. The product is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies may be generated for subsequent use in immunoassays

10    to measure the protein.

[115]    Methods of production of polyclonal antibodies are known to those of skill in the art. In brief, an immunogen, preferably a purified protein, is mixed with an adjuvant and animals are immunized. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to

15    polypeptides of the invention. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be done if desired (*see*, Harlow and Lane, *supra*).

[116]    Monoclonal antibodies may be obtained using various techniques

20    familiar to those of skill in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell (*see*, Kohler and Milstein, *Eur. J. Immunol.* 6:511-519 (1976)). Alternative methods of immortalization include, *e.g.*, transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods well known in the art. Colonies arising from single immortalized cells are screened

25    for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences that encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according to the general protocol outlined by

30    Huse *et al.*, *supra*.

[117]    Once target immunogen-specific antibodies are available, the immunogen can be measured by a variety of immunoassay methods with qualitative and quantitative results available to the clinician. For a review of immunological and immunoassay procedures in general *see*, Stites, *supra*. Moreover, the immunoassays of the

present invention can be performed in any of several configuration, which are reviewed

extensively in Maggio *Enzyme Immunoassay*, CRC Press, Boca Raton, Florida (1980);

Tijssen, *supra*; and Harlow and Lane, *supra*.

[118]    Immunoassays to measure target proteins in a human sample may use

a polyclonal antiserum that was raised to full-length polypeptides of the invention or a

fragment thereof. This antiserum is selected to have low cross-reactivity against other

proteins and any such cross-reactivity is removed by immunoabsorption prior to use in the

immunoassay.

**B.    Immunological Binding Assays**

[119]    In some embodiments, a protein of interest is detected and/or

quantified using any of a number of well-known immunological binding assays (*see, e.g.,*

U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of the general

immunoassays, *see* also Asai *Methods in Cell Biology Volume 37: Antibodies in Cell*

*Biology*, Academic Press, Inc. NY (1993); Stites, *supra*. Immunological binding assays (or

immunoassays) typically utilize a "capture agent" to specifically bind to and often

immobilize the analyte (e.g., full-length polypeptides of the present invention, or antigenic

subsequences thereof). The capture agent is a moiety that specifically binds to the analyte.

The antibody may be produced by any of a number of means well known to those of skill in

the art and as described above.

[120]    Immunoassays also often utilize a labeling agent to bind specifically to

and label the binding complex formed by the capture agent and the analyte. The labeling

agent may itself be one of the moieties comprising the antibody/analyte complex.

Alternatively, the labeling agent may be a third moiety, such as another antibody, that

specifically binds to the antibody/protein complex.

[121]    In a preferred embodiment, the labeling agent is a second antibody

bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be

bound by a labeled third antibody specific to antibodies of the species from which the second

antibody is derived. The second antibody can be modified with a detectable moiety, such as

biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled

streptavidin.

[122]    Other proteins capable of specifically binding immunoglobulin

constant regions, such as protein A or protein G, can also be used as the label agents. These

proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (*see*, generally, Kronval, *et al. J. Immunol.*, 111:1401-1406 (1973); and Akerstrom, *et al. J. Immunol.*, 135:2589-2542 (1985)).

5          [123]   Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. The incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be

10    conducted over a range of temperatures, such as 10°C to 40°C.

### 1. Non-Competitive Assay Formats

[124]   Immunoassays for detecting proteins or analytes of interest from tissue samples may be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured protein or analyte is directly measured. In one

15    preferred "sandwich" assay, for example, the capture agent (*e.g.,* antibodies specific for the polypeptides of the invention) can be bound directly to a solid substrate where it is immobilized. These immobilized antibodies then capture the polypeptide present in the test sample. The polypeptide of the invention thus immobilized is then bound by a labeling agent, such as a second labelled antibody specific for the polypeptide. Alternatively, the

20    second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

### 2. Competitive Assay Formats

25          [125]   In competitive assays, the amount of protein or analyte present in the sample is measured indirectly by measuring the amount of an added (exogenous) protein or analyte displaced (or competed away) from a specific capture agent (e.g., antibodies specific for a polypeptide of the invention) by the protein or analyte present in the sample. The amount of immunogen bound to the antibody is inversely proportional to the concentration of

30    immunogen present in the sample. In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. The amount of analyte may be detected by providing a labeled analyte molecule. It is understood that labels can include, e.g., radioactive labels as well as peptide or other tags that can be recognized by detection reagents such as antibodies.

[126]  Immunoassays in the competitive binding that can be used for cross-reactivity determinations.  For example, the protein encoded by the sequences described herein can be immobilized on a solid support.  Proteins are added to the assay and compete with the binding of the antisera to the immobilized antigen.  The ability of the above proteins

5    to compete with the binding of the antisera to the immobilized protein is compared to that of the protein encoded by any of the sequences described herein.  The percent cross-reactivity for the above proteins is calculated, using standard calculations.  Those antisera with less than 10% cross-reactivity with each of the proteins listed above are selected and pooled.  The cross-reacting antibodies are optionally removed from the pooled antisera by

10   immunoabsorption with the considered proteins, *e.g.,* distantly related homologs.

[127]  The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein, thought to be perhaps a protein of the present invention, to the immunogen protein.  In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the

15   amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined.  If the amount of the second protein required is less than 10 times the amount of the protein partially encoded by a sequence herein that is required, then the second protein is said to specifically bind to an antibody generated to an immunogen consisting of the target protein.

20   <u>3.  Other Assay Formats</u>

[128]  In some embodiments, western blot (immunoblot) analysis is used to detect and quantify the presence of a polypeptide of the invention in the sample.  The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support (such as,

25   *e.g.,* a nitrocellulose filter, a nylon filter, or a derivatized nylon filter) and incubating the sample with the antibodies that specifically bind the protein of interest.  For example, antibodies are selected that specifically bind to the polypeptides of the invention on the solid support.  These antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (*e.g.,* labeled sheep anti-mouse antibodies) that specifically

30   bind to the antibodies against the protein of interest.

[129]  Other assay formats include liposome immunoassays (LIA), which use liposomes designed to bind specific molecules (*e.g.,* antibodies) and release encapsulated reagents or markers.  The released chemicals are then detected according to standard techniques (*see,* Monroe *et al.* (1986) *Amer. Clin. Prod. Rev.* 5:34-41).

## 4. Labels

[130]   The particular label or detectable group used in the assay is not a critical aspect of the invention, as long as it does not significantly interfere with the specific binding of the antibody used in the assay.  The detectable group can be any material having a detectable physical or chemical property.  Such detectable labels have been well-developed in the field of immunoassays and, in general, most labels useful in such methods can be applied to the present invention.  Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means.  Useful labels in the present invention include magnetic beads (*e.g.,* Dynabeads$^{TM}$), fluorescent dyes (*e.g.,* fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.,* $^{3}$H, $^{125}$I, $^{35}$S, $^{14}$C, or $^{32}$P), enzymes (*e.g.,* horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (*e.g.,* polystyrene, polypropylene, latex, *etc.*) beads.

[131]   The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art.  As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, the ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[132]   Non-radioactive labels are often attached by indirect means.  The molecules can also be conjugated directly to signal generating compounds, *e.g.,* by conjugation with an enzyme or fluorescent compound.  A variety of enzymes and fluorescent compounds can be used with the methods of the present invention and are well-known to those of skill in the art (for a review of various labeling or signal producing systems which may be used, *see, e.g.,* U.S. Patent No. 4,391,904).

[133]   Means of detecting labels are well known to those of skill in the art.  Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography.  Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence.  The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like.  Similarly, enzymatic labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product.  Finally simple colorimetric labels may be detected directly by

observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[134] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need to be labeled and the presence of the target antibody is detected by simple visual inspection.

## VI.    IDENTIFICATION OF MODULATORS OF POLYPEPTIDES OF THE INVENTION

[135] Modulators of a polypeptide of the invention, i.e. agonists or antagonists of a polypeptide's activity, or polypeptide's or polynucleotide's expression, are useful for treating a number of human diseases, including diabetes. For example, administration of modulators can be used to treat diabetic patients or prediabetic individuals to prevent progression, and therefore symptoms, associated with diabetes (including insulin resistance).

### A.    Agents that Modulate Polypeptides of the Invention

[136] The agents tested as modulators of polypeptides of the invention can be any small chemical compound, or a biological entity, such as a protein, sugar, nucleic acid or lipid. Typically, test compounds will be small chemical molecules and peptides. Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although most often compounds that can be dissolved in aqueous or organic (especially DMSO-based) solutions are used. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (*e.g.*, in microtiter formats on microtiter plates in robotic assays). Modulators also include agents designed to reduce the level of mRNA encoding a polypeptide of the invention (e.g. antisense molecules, ribozymes, DNAzymes, small inhibitory RNAs and the like) or the level of translation from an mRNA (e.g., translation blockers such as an antisense molecules that are complementary to translation start or other sequences on an mRNA molecule). It will be appreciated that there are many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland) and the like.

[137]    In some embodiments, high throughput screening methods involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (potential modulator compounds). Such "combinatorial chemical libraries" or "ligand libraries" are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as conventional "lead compounds" or can themselves be used as potential or actual therapeutics.

[138]    A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (*i.e.*, the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

[139]    Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art. Such combinatorial chemical libraries include, but are not limited to, peptide libraries (*see, e.g.,* U.S. Patent 5,010,175, Furka, *Int. J. Pept. Prot. Res.* 37:487-493 (1991) and Houghton *et al., Nature* 354:84-88 (1991)). Other chemistries for generating chemical diversity libraries can also be used. Such chemistries include, but are not limited to: peptoids (*e.g.,* PCT Publication No. WO 91/19735), encoded peptides (*e.g.,* PCT Publication WO 93/20242), random bio-oligomers (*e.g.,* PCT Publication No. WO 92/00091), benzodiazepines (*e.g.,* U.S. Pat. No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs *et al., Proc. Nat. Acad. Sci. USA* 90:6909-6913 (1993)), vinylogous polypeptides (Hagihara *et al., J. Amer. Chem. Soc.* 114:6568 (1992)), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann *et al., J. Amer. Chem. Soc.* 114:9217-9218 (1992)), analogous organic syntheses of small compound libraries (Chen *et al., J. Amer. Chem. Soc.* 116:2661 (1994)), oligocarbamates (Cho *et al., Science* 261:1303 (1993)), and/or peptidyl phosphonates (Campbell *et al., J. Org. Chem.* 59:658 (1994)), nucleic acid libraries (*see* Ausubel, Berger and Sambrook, all *supra*), peptide nucleic acid libraries (*see, e.g.,* U.S. Patent 5,539,083), antibody libraries (*see, e.g.,* Vaughn *et al., Nature Biotechnology,* 14(3):309-314 (1996) and PCT/US96/10287), carbohydrate libraries (*see, e.g.,* Liang *et al., Science,* 274:1520-1522 (1996) and U.S. Patent 5,593,853), small organic molecule libraries (*see, e.g.,* benzodiazepines, Baum C&EN, Jan 18, page 33 (1993); isoprenoids, U.S. Patent 5,569,588; thiazolidinones and metathiazanones, U.S. Patent

5,549,974; pyrrolidines, U.S. Patents 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent 5,506,337; benzodiazepines, 5,288,514, and the like).

[140]   Devices for the preparation of combinatorial libraries are commercially available (*see, e.g.,* 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY, Symphony, Rainin, Woburn, MA, 433A Applied Biosystems, Foster City, CA, 9050 Plus, Millipore, Bedford, MA). In addition, numerous combinatorial libraries are themselves commercially available (*see, e.g.,* ComGenex, Princeton, N.J., Tripos, Inc., St. Louis, MO, 3D Pharmaceuticals, Exton, PA, Martek Biosciences, Columbia, MD, etc.).

## B.    Methods of Screening for Modulators of the Polypeptides of the Invention

[141]   A number of different screening protocols can be utilized to identify agents that modulate the level of expression or activity of a polynucleotide of a polypeptide of the invention in cells, particularly mammalian cells, and especially human cells. In general terms, the screening methods involve screening a plurality of agents to identify an agent that modulates the activity of a polypeptide of the invention by, e.g., binding to the polypeptide, preventing an inhibitor or activator from binding to the polypeptide, increasing association of an inhibitor or activator with the polypeptide, or activating or inhibiting expression of the polypeptide.

[142]   Any cell expressing a full-length polypeptide of the invention or a fragment thereof can be used to identify modulators. In some embodiments, the cells are eukaryotic cells lines (e.g., CHO or HEK293) transformed to express a heterologous polypeptide of the invention. In some embodiments, a cell expressing an endogenous polypeptide of the invention is used in screens. In other embodiments, modulators are screened for their ability to effect insulin responses.

### 1.    Polypeptide Binding Assays

[143]   Preliminary screens can be conducted by screening for agents capable of binding to polypeptides of the invention, as at least some of the agents so identified are likely modulators of a polypeptide of the invention. Binding assays are also useful, e.g., for identifying endogenous proteins that interact with polypeptides of the invention. For example, antibodies, receptors or other molecules that bind polypeptides of the invention can be identified in binding assays.

[144]    Binding assays usually involve contacting a polypeptide of the
invention with one or more test agents and allowing sufficient time for the protein and test
agents to form a binding complex. Any binding complexes formed can be detected using any
of a number of established analytical techniques. Protein binding assays include, but are not
5    limited to, methods that measure co-precipitation or co-migration on non-denaturing SDS-
polyacrylamide gels, and co-migration on Western blots (see, e.g., Bennet, J.P. and
Yamamura, H.I. (1985) "Neurotransmitter, Hormone or Drug Receptor Binding Methods," in
Neurotransmitter Receptor Binding (Yamamura, H. I., et al., eds.), pp. 61-89. Other binding
assays involve the use of mass spectrometry or NMR techniques to identify molecules bound
10    to a polypeptide of the invention or displacement of labeled substrates. The polypeptides of
the invention utilized in such assays can be naturally expressed, cloned or synthesized.

[145]    In addition, mammalian or yeast two-hybrid approaches (see, e.g.,
Bartel, P.L. et. al. Methods Enzymol, 254:241 (1995)) can be used to identify polypeptides or
other molecules that interact or bind when expressed together in a host cell.

15

2.    Polypeptide Activity

[146]    The activity of polypeptides of the invention can be assessed using a
variety of in vitro and in vivo assays to determine functional, chemical, and physical effects,
e.g., measuring ligand binding (e.g., radioactive or otherwise labeled ligand binding), second
20    messengers (e.g., cAMP, cGMP, $IP_3$, DAG, or $Ca^{2+}$), ion flux, phosphorylation levels,
transcription levels, and the like. Furthermore, such assays can be used to test for inhibitors
and activators of the polypeptides of the invention. Modulators can also be genetically
altered versions of polypeptides of the invention.

[147]    The polypeptide of the assay will be selected from a polypeptide with
25    substantial identity to a sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID
NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14 or other conservatively modified
variants thereof. Generally, the amino acid sequence identity will be at least 70%, optionally
at least 85%, optionally at least 90-95% to the polypeptides exemplified herein. Optionally,
the polypeptide of the assays will comprise a fragment of a polypeptide of the invention, such
30    as an extracellular domain, transmembrane domain, cytoplasmic domain, ligand binding
domain, subunit association domain, active site, and the like. Either a polypeptide of the
invention or a domain thereof can be covalently linked to a heterologous protein to create a
chimeric protein used in the assays described herein.

[148] Modulators of polypeptide activity are tested using either recombinant or naturally occurring polypeptides of the invention. The protein can be isolated, expressed in a cell, expressed in a membrane derived from a cell, expressed in tissue or in an animal, either recombinant or naturally occurring. For example, tissue slices, dissociated cells, e.g., from tissues expressing polypeptides of the invention, transformed cells, or membranes can be used. Modulation is tested using one of the *in vitro* or *in vivo* assays described herein.

[149] Modulator binding to polypeptides of the invention, a domain, or chimeric protein can be tested in solution, in a bilayer membrane, attached to a solid phase, in a lipid monolayer, or in vesicles. Binding of a modulator can be tested using, *e.g.*, changes in spectroscopic characteristics (*e.g.*, fluorescence, absorbance, refractive index), hydrodynamic (*e.g.*, shape), chromatographic, or solubility properties.

[150] Samples or assays that are treated with a potential moulator (e.g., a "test compound") are compared to control samples without the test compound, to examine the extent of modulation. Control samples (untreated with activators or inhibitors) are assigned a relative activity value of 100. Inhibition of the polypeptides of the invention is achieved when the activity value relative to the control is about 90%, optionally 50%, optionally 25-0%. Activation of the polypeptides of the invention is achieved when the activity value relative to the control is 110%, optionally 150%, 200%, 300%, 400%, 500%, or 1000-2000%.

### 3.    Expression Assays

[151] Screening for a compound that modulates the expression of a polynucleotide or a polypeptide of the invention is also provided. Screening methods generally involve conducting cell-based assays in which test compounds are contacted with one or more cells expressing a polynucleotide or a polypeptide of the invention, and then detecting an increase or decrease in expression (either transcript or translation product). Assays can be performed with any cells that express a polynucleotide or a polypeptide of the invention.

[152] Expression can be detected in a number of different ways. As described *infra*, the expression level of a polynucleotide of the invention in a cell can be determined by probing the mRNA expressed in a cell with a probe that specifically hybridizes with a transcript (or complementary nucleic acid derived therefrom) of a polynucleotide of the invention. Probing can be conducted by lysing the cells and conducting Northern blots or without lysing the cells using *in situ*-hybridization techniques. Alternatively, a polypeptide

of the invention can be detected using immunological methods in which a cell lysate is probed with antibodies that specifically bind to the polypeptide.

[153] The level of expression or activity of a polynucleotide or a polypeptide of the invention can be compared to a baseline value. The baseline value can be a value for a control sample or a statistical value that is representative of expression levels of a polynucleotide or a polypeptide of the invention for a control population (e.g., lean individuals as described herein) or cells (e.g., tissue culture cells not exposed to a modulator). Expression levels can also be determined for cells that do not express the polynucleotide or a polypeptide of the invention as a negative control. Such cells generally are otherwise substantially genetically the same as the test cells.

[154] A variety of different types of cells can be utilized in the reporter assays. Cells that do not endogenously express a polypeptide of the invention can be prokaryotic, but are preferably eukaryotic. The eukaryotic cells can be any of the cells typically utilized in generating cells that harbor recombinant nucleic acid constructs. Exemplary eukaryotic cells include, but are not limited to, yeast, and various higher eukaryotic cells such as the HEK293, HepG2, COS, CHO and HeLa cell lines.

[155] Various controls can be conducted to ensure that an observed activity is authentic including running parallel reactions with cells that lack the reporter construct or by not contacting a cell harboring the reporter construct with test compound. Compounds can also be further validated as described below.

4.    Validation

[156] Agents that are initially identified by any of the foregoing screening methods can be further tested to validate the apparent activity. Modulators that are selected for further study can be tested on the "classic" insulin responsive cell line, mouse 3T3-L1 adipocytes, muscle cells such as L6 cells and the like. Cells (e.g., adipocytes or muscle cells) are pre-incubated with the modulators and tested for acute (up to 4 hours) and chronic (overnight) effects on basal and insulin-stimulated GLUT4 translocation and glucose uptake.

[157] Following such studies, validity of the modulators is tested in suitable animal models. The basic format of such methods involves administering a lead compound identified during an initial screen to an animal that serves as a model for humans and then determining if expressionof activity of a polypeptide of the invention is in fact modulated.

[158] The effect of the compound will be assessed in either diabetic animals or in diet induced insulin resistant animals. The blood glucose and insulin levels will be

determined. The animal models utilized in validation studies generally are mammals of any

kind. Specific examples of suitable animals include, but are not limited to, primates, mice

and rats. For example, monogenic models of diabetes (e.g., ob/ob and db/db mice, Zucker

rats and Zucker Diabetic Fatty rats etc) or polygenic models of diabetes (e.g., OLETF rats,

5        GK rats, NSY mice, and KK mice) can be useful for validating modulation of a polypeptide

of the invention in a diabetic or insulin resistant animal. In addition, transgenic animals

expressing human polypeptides of the invention can be used to further validate drug

candidates.


10        **C.     Solid Phase and Soluble High Throughput Assays**

         [159]    In the high throughput assays of the invention, it is possible to screen

up to several thousand different modulators or ligands in a single day. In particular, each

well of a microtiter plate can be used to run a separate assay against a selected potential

modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells

15        can test a single modulator. Thus, a single standard microtiter plate can assay about 100

(e.g., 96) modulators. If 1536 well plates are used, then a single plate can easily assay from

about 100 to about 1500 different compounds. It is possible to assay several different plates

per day; assay screens for up to about 6,000-20,000 or more different compounds are possible

using the integrated systems of the invention. In addition, microfluidic approaches to reagent

20        manipulation can be used.

         [160]    A molecule of interest (e.g., a polypeptide or polynucleotide of the

invention, or a modulator thereof) can be bound to the solid-state component, directly or

indirectly, via covalent or non-covalent linkage, e.g., via a tag. The tag can be any of a

variety of components. In general, a molecule that binds the tag (a tag binder) is fixed to a

25        solid support, and the tagged molecule of interest is attached to the solid support by

interaction of the tag and the tag binder.

         [161]    A number of tags and tag binders can be used, based upon known

molecular interactions well described in the literature. For example, where a tag has a natural

binder, for example, biotin, protein A, or protein G, it can be used in conjunction with

30        appropriate tag binders (avidin, streptavidin, neutravidin, the Fc region of an

immunoglobulin, poly-His, etc.) Antibodies to molecules with natural binders such as biotin

are also widely available and appropriate tag binders (see, SIGMA Immunochemicals 1998

catalogue SIGMA, St. Louis MO).

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[162]    Similarly, any haptenic or antigenic compound can be used in combination with an appropriate antibody to form a tag/tag binder pair. Thousands of specific antibodies are commercially available and many additional antibodies are described in the literature. For example, in one common configuration, the tag is a first antibody and

5      the tag binder is a second antibody that recognizes the first antibody. In addition to antibody-antigen interactions, receptor-ligand interactions are also appropriate as tag and tag-binder pairs, such as agonists and antagonists of cell membrane receptors (*e.g.,* cell receptor-ligand interactions such as transferrin, c-kit, viral receptor ligands, cytokine receptors, chemokine receptors, interleukin receptors, immunoglobulin receptors and antibodies, the cadherin

10     family, the integrin family, the selectin family, and the like; *see, e.g.,* Pigott & Power, *The Adhesion Molecule Facts Book I* (1993)). Similarly, toxins and venoms, viral epitopes, hormones (*e.g.,* opiates, steroids, *etc.*), intracellular receptors (*e.g.,* which mediate the effects of various small ligands, including steroids, thyroid hormone, retinoids and vitamin D; peptides), drugs, lectins, sugars, nucleic acids (both linear and cyclic polymer

15     configurations), oligosaccharides, proteins, phospholipids and antibodies can all interact with various cell receptors.

[163]    Synthetic polymers, such as polyurethanes, polyesters, polycarbonates, polyureas, polyamides, polyethyleneimines, polyarylene sulfides, polysiloxanes, polyimides, and polyacetates can also form an appropriate tag or tag binder. Many other tag/tag binder

20     pairs are also useful in assay systems described herein, as would be apparent to one of skill upon review of this disclosure.

[164]    Common linkers such as peptides, polyethers, and the like can also serve as tags, and include polypeptide sequences, such as poly-gly sequences of between about 5 and 200 amino acids. Such flexible linkers are known to those of skill in the art. For

25     example, poly(ethelyne glycol) linkers are available from Shearwater Polymers, Inc., Huntsville, Alabama. These linkers optionally have amide linkages, sulfhydryl linkages, or heterofunctional linkages.

[165]    Tag binders are fixed to solid substrates using any of a variety of methods currently available. Solid substrates are commonly derivatized or functionalized by

30     exposing all or a portion of the substrate to a chemical reagent that fixes a chemical group to the surface that is reactive with a portion of the tag binder. For example, groups that are suitable for attachment to a longer chain portion would include amines, hydroxyl, thiol, and carboxyl groups. Aminoalkylsilanes and hydroxyalkylsilanes can be used to functionalize a variety of surfaces, such as glass surfaces. The construction of such solid phase biopolymer

arrays is well described in the literature (*see, e.g.*, Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963) (describing solid phase synthesis of, *e.g.*, peptides); Geysen *et al., J. Immun. Meth.* 102:259-274 (1987) (describing synthesis of solid phase components on pins); Frank and Doring, *Tetrahedron* 44:60316040 (1988) (describing synthesis of various peptide sequences on cellulose disks); Fodor *et al., Science,* 251:767-777 (1991); Sheldon *et al., Clinical Chemistry* 39(4):718-719 (1993); and Kozal *et al., Nature Medicine* 2(7):753759 (1996) (all describing arrays of biopolymers fixed to solid substrates). Non-chemical approaches for fixing tag binders to substrates include other common methods, such as heat, cross-linking by UV radiation, and the like.

[166]    The invention provides *in vitro* assays for identifying, in a high throughput format, compounds that can modulate the expression or activity of a polypeptide of the invention. Control reactions that measure activity of a polypeptide of the invention in a cell in a reaction that does not include a potential modulator are optional, as the assays are highly uniform. Such optional control reactions are appropriate and increase the reliability of the assay. Accordingly, in some embodiments, the methods of the invention include such a control reaction. For each of the assay formats described, "no modulator" control reactions that do not include a modulator provide a background level of binding activity.

[167]    In some assays it will be desirable to have positive controls. At least two types of positive controls are appropriate. First, a known activator of a polypeptide or a polynucleotide of the invention can be incubated with one sample of the assay, and the resulting increase in signal resulting from an increased expression level or activity of a polypeptide or a polynucleotide of the invention are determined according to the methods herein. Second, a known inhibitor of a polypeptide or a polynucleotide of the invention can be added, and the resulting decrease in signal for the expression or activity of a polypeptide or a polynucleotide of the invention can be similarly detected. It will be appreciated that modulators can also be combined with activators or inhibitors to find modulators that inhibit the increase or decrease that is otherwise caused by the presence of the known modulator of a polypeptide or a polynucleotide of the invention.

## VII.    COMPOSITIONS, KITS AND INTEGRATED SYSTEMS

[168]    The invention provides compositions, kits and integrated systems for practicing the assays described herein using nucleic acids or polypeptides of the invention, antibodies, etc.

[169]   The invention provides assay compositions for use in solid phase assays; such compositions can include, for example, one or more nucleic acids encoding a polypeptide of the invention immobilized on a solid support, and a labeling reagent. In each case, the assay compositions can also include additional reagents that are desirable for

5       hybridization. Modulators of expression or activity of a polypeptide of the invention can also be included in the assay compositions.

[170]   The invention also provides kits for carrying out the assays of the invention. The kits typically include a probe that comprises an antibody that specifically binds to a polypeptide of the invention or a polynucleotide sequence encoding such

10      polypeptides, and a label for detecting the presence of the probe. The kits may include at least one polynucleotide sequence encoding a polypeptide of the invention. Kits can include any of the compositions noted above, and optionally further include additional components such as instructions to practice a high-throughput method of assaying for an effect on expression of the genes encoding a polypeptide of the invention, or on activity of a

15      polypeptide of the invention, one or more containers or compartments (e.g., to hold the probe, labels, or the like), a control modulator of the expression or activity of a polypeptide of the invention, a robotic armature for mixing kit components or the like.

[171]   The invention also provides integrated systems for high-throughput screening of potential modulators for an effect on the expression or activity of a polypeptide

20      of the invention. The systems can include a robotic armature which transfers fluid from a source to a destination, a controller which controls the robotic armature, a label detector, a data storage unit which records label detection, and an assay component such as a microtiter dish comprising a well having a reaction mixture or a substrate comprising a fixed nucleic acid or immobilization moiety.

25      [172]   A number of robotic fluid transfer systems are available, or can easily be made from existing components. For example, a Zymate XP (Zymark Corporation; Hopkinton, MA) automated robot using a Microlab 2200 (Hamilton; Reno, NV) pipetting station can be used to transfer parallel samples to 96 well microtiter plates to set up several parallel simultaneous binding assays.

30      [173]   Optical images viewed (and, optionally, recorded) by a camera or other recording device (e.g., a photodiode and data storage device) are optionally further processed in any of the embodiments herein, e.g., by digitizing the image and storing and analyzing the image on a computer. A variety of commercially available peripheral equipment and

software is available for digitizing, storing and analyzing a digitized video or digitized optical image.

[174]   One conventional system carries light from the specimen field to a cooled charge-coupled device (CCD) camera, in common use in the art. A CCD camera

5   includes an array of picture elements (pixels). The light from the specimen is imaged on the CCD. Particular pixels corresponding to regions of the specimen (*e.g.,* individual hybridization sites on an array of biological polymers) are sampled to obtain light intensity readings for each position. Multiple pixels are processed in parallel to increase speed. The apparatus and methods of the invention are easily used for viewing any sample, *e.g.,* by

10   fluorescent or dark field microscopic techniques.

## VIII. ADMINISTRATION AND PHARMACEUTICAL COMPOSITIONS

[175]   Modulators of the polypeptides of the invention (e.g., antagonists or agonists) can be administered directly to the mammalian subject for modulation of activity of a polypeptide of the invention *in vivo.* Administration is by any of the routes normally used

15   for introducing a modulator compound into ultimate contact with the tissue to be treated and is well known to those of skill in the art. Although more than one route can be used to administer a particular composition, a particular route can often provide a more immediate and more effective reaction than another route.

[176]   The pharmaceutical compositions of the invention may comprise a

20   pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g., Remington's Pharmaceutical Sciences,* 17th ed. 1985)).

25   [177]   The modulators (e.g., agonists or antagonists) of the expression or activity of the a polypeptide of the invention, alone or in combination with other suitable components, can be prepared for injection or for use in a pump device. Pump devices (also known as "insulin pumps") are commonly used to administer insulin to patients and therefore can be easily adapted to include compositions of the present invention. Manufacturers of

30   insulin pumps include Animas, Disetronic and MiniMed.

[178]   The modulators (e.g., agonists or antagonists) of the expression or activity of a polypeptide of the invention, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.,* they can be "nebulized") to be

administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

[179] Formulations suitable for administration include aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, orally, nasally, topically, intravenously, intraperitoneally, or intrathecally. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials. Solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. The modulators can also be administered as part of a prepared food or drug.

[180] The dose administered to a patient, in the context of the present invention should be sufficient to induce a beneficial response in the subject over time. The optimal dose level for any patient will depend on a variety of factors including the efficacy of the specific modulator employed, the age, body weight, physical activity, and diet of the patient, on a possible combination with other drugs, and on the severity of the case of diabetes. It is recommended that the daily dosage of the modulator be determined for each individual patient by those skilled in the art in a similar way as for known insulin compositions. The size of the dose also will be determined by the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound or vector in a particular subject.

[181] In determining the effective amount of the modulator to be administered a physician may evaluate circulating plasma levels of the modulator, modulator toxicity, and the production of anti-modulator antibodies. In general, the dose equivalent of a modulator is from about 1 ng/kg to 10 mg/kg for a typical subject.

[182] For administration, modulators of the present invention can be administered at a rate determined by the LD-50 of the modulator, and the side-effects of the modulator at various concentrations, as applied to the mass and overall health of the subject. Administration can be accomplished via single or divided doses.

[183] The compounds of the present invention can also be used effectively in combination with one or more additional active agents depending on the desired target therapy (see, e.g., Turner, N. et al. *Prog. Drug Res.* (1998) 51: 33-94; Haffner, S. *Diabetes Care* (1998) 21: 160-178; and DeFronzo, R. et al. (eds.), *Diabetes Reviews* (1997) Vol. 5 No.

4). A number of studies have investigated the benefits of combination therapies with oral

agents (see, e.g., Mahler, R., *J. Clin. Endocrinol. Metab.* (1999) 84: 1165-71; United

Kingdom Prospective Diabetes Study Group: UKPDS 28, *Diabetes Care* (1998) 21: 87-92;

Bardin, C. W.,(ed.), *Current Therapy In Endocrinology And Metabolism*, 6th Edition (Mosby

5   - Year Book, Inc., St. Louis, MO 1997); Chiasson, J. et al., *Ann. Intern. Med.* (1994) 121:

928-935; Coniff, R. et al., *Clin. Ther.* (1997) 19: 16-26; Coniff, R. et al., *Am. J. Med.* (1995)

98: 443-451; and Iwamoto, Y. et al., *Diabet. Med.* (1996) 13 365-370; Kwiterovich, P. *Am. J.*

*Cardiol* (1998) 82(12A): 3U-17U). These studies indicate that modulation of diabetes,

among other diseases, can be further improved by the addition of a second agent to the

10  therapeutic regimen. Combination therapy includes administration of a single pharmaceutical

dosage formulation that contains a modulator of the invention and one or more additional

active agents, as well as administration of a modulator and each active agent in its own

separate pharmaceutical dosage formulation. For example, a modulator and a

thiazolidinedione can be administered to the human subject together in a single oral dosage

15  composition, such as a tablet or capsule, or each agent can be administered in separate oral

dosage formulations. Where separate dosage formulations are used, a modulator and one or

more additional active agents can be administered at essentially the same time (i.e.,

concurrently), or at separately staggered times (i.e., sequentially). Combination therapy is

understood to include all these regimens.

20          [184]   One example of combination therapy can be seen in treating pre-

diabetic individuals (e.g., to prevent progression into type 2 diabetes) or diabetic individuals

(or treating diabetes and its related symptoms, complications, and disorders), wherein the

modulators can be effectively used in combination with, for example, sulfonylureas (such as

chlorpropamide, tolbutamide, acetohexamide, tolazamide, glyburide, gliclazide, glynase,

25  glimepiride, and glipizide); biguanides (such as metformin); a PPAR beta delta agonist; a

ligand or agonist of PPAR gamma such as thiazolidinediones (such as ciglitazone,

pioglitazone (*see, e.g.*, U.S. Patent No. 6,218,409), troglitazone, and rosiglitazone (*see, e.g.*,

U.S. Patent No. 5,859,037)); PPAR alpha agonists such as clofibrate, gemfibrozil,

fenofibrate, ciprofibrate, and bezafibrate; dehydroepiandrosterone (also referred to as DHEA

30  or its conjugated sulphate ester, DHEA-SO4); antiglucocorticoids; TNFα inhibitors; α-

glucosidase inhibitors (such as acarbose, miglitol, and voglibose); amylin and amylin

derivatives (such as pramlintide, (*see, also*, U.S. Patent Nos. 5,902,726; 5,124,314; 5,175,145

and 6,143,718.)); insulin secretogogues (such as repaglinide, gliquidone, and nateglinide (*see,*

*also*, U.S. Patent Nos. 6,251,856; 6,251,865; 6,221,633; 6,174,856)), and insulin.

## IX.  GENE THERAPY

[185]  Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids encoding engineered polypeptides of the invention in mammalian cells or target tissues.  Such methods can be used to administer nucleic acids encoding polypeptides of the invention to cells *in vitro*.  In some embodiments, the nucleic acids encoding polypeptides of the invention are administered for *in vivo* or *ex vivo* gene therapy uses.  Non-viral vector delivery systems include DNA plasmids, naked nucleic acid, and nucleic acid complexed with a delivery vehicle such as a liposome.  Viral vector delivery systems include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell.  For a review of gene therapy procedures, see Anderson, *Science* 256:808-813 (1992); Nabel & Felgner, *TIBTECH* 11:211-217 (1993); Mitani & Caskey, *TIBTECH* 11:162-166 (1993); Dillon, *TIBTECH* 11:167-175 (1993); Miller, *Nature* 357:455-460 (1992); Van Brunt, *Biotechnology* 6(10):1149-1154 (1988); Vigne, *Restorative Neurology and Neuroscience* 8:35-36 (1995); Kremer & Perricaudet, *British Medical Bulletin* 51(1):31-44 (1995); Haddada *et al.*, in *Current Topics in Microbiology and Immunology* Doerfler and Böhm (eds) (1995); and Yu *et al., Gene Therapy* 1:13-26 (1994).

[186]  Methods of non-viral delivery of nucleic acids encoding engineered polypeptides of the invention include lipofection, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid:nucleic acid conjugates, naked DNA, artificial virions, and agent-enhanced uptake of DNA.  Lipofection is described in e.g., US 5,049,386, US 4,946,787; and US 4,897,355) and lipofection reagents are sold commercially (e.g., Transfectam™ and Lipofectin™).  Cationic and neutral lipids that are suitable for efficient receptor-recognition lipofection of polynucleotides include those of Felgner, WO 91/17424, WO 91/16024.  Delivery can be to cells (*ex vivo* administration) or target tissues (*in vivo* administration).

[187]  The preparation of lipid:nucleic acid complexes, including targeted liposomes such as immunolipid complexes, is well known to one of skill in the art (*see, e.g.,* Crystal, *Science* 270:404-410 (1995); Blaese *et al., Cancer Gene Ther.* 2:291-297 (1995); Behr *et al., Bioconjugate Chem.* 5:382-389 (1994); Remy *et al., Bioconjugate Chem.* 5:647-654 (1994); Gao *et al., Gene Therapy* 2:710-722 (1995); Ahmad *et al., Cancer Res.* 52:4817-4820 (1992); U.S. Pat. Nos. 4,186,183, 4,217,344, 4,235,871, 4,261,975, 4,485,054, 4,501,728, 4,774,085, 4,837,028, and 4,946,787).

[188]  The use of RNA or DNA viral based systems for the delivery of nucleic acids encoding engineered polypeptides of the invention take advantage of highly

evolved processes for targeting a virus to specific cells in the body and trafficking the viral

payload to the nucleus. Viral vectors can be administered directly to patients (*in vivo*) or they

can be used to treat cells *in vitro* and the modified cells are administered to patients (*ex vivo*).

Conventional viral based systems for the delivery of polypeptides of the invention could

5      include retroviral, lentivirus, adenoviral, adeno-associated and herpes simplex virus vectors

for gene transfer. Viral vectors are currently the most efficient and versatile method of gene

transfer in target cells and tissues. Integration in the host genome is possible with the

retrovirus, lentivirus, and adeno-associated virus gene transfer methods, often resulting in

long term expression of the inserted transgene. Additionally, high transduction efficiencies

10     have been observed in many different cell types and target tissues.

[189] The tropism of a retrovirus can be altered by incorporating foreign

envelope proteins, expanding the potential target population of target cells. Lentiviral vectors

are retroviral vectors that are able to transduce or infect non-dividing cells and typically

produce high viral titers. Selection of a retroviral gene transfer system would therefore

15     depend on the target tissue. Retroviral vectors are comprised of *cis*-acting long terminal

repeats with packaging capacity for up to 6-10 kb of foreign sequence. The minimum *cis*-

acting LTRs are sufficient for replication and packaging of the vectors, which are then used

to integrate the therapeutic gene into the target cell to provide permanent transgene

expression. Widely used retroviral vectors include those based upon murine leukemia virus

20     (MuLV), gibbon ape leukemia virus (GaLV), Simian Immuno deficiency virus (SIV), human

immuno deficiency virus (HIV), and combinations thereof (*see, e.g.,* Buchscher *et al., J.*

*Virol.* 66:2731-2739 (1992); Johann *et al., J. Virol.* 66:1635-1640 (1992); Sommerfelt *et al.,*

*Virol.* 176:58-59 (1990); Wilson *et al., J. Virol.* 63:2374-2378 (1989); Miller *et al., J. Virol.*

65:2220-2224 (1991); PCT/US94/05700).

25     [190] In applications where transient expression of the polypeptides of the

invention is preferred, adenoviral based systems are typically used. Adenoviral based vectors

are capable of very high transduction efficiency in many cell types and do not require cell

division. With such vectors, high titer and levels of expression have been obtained. This

vector can be produced in large quantities in a relatively simple system. Adeno-associated

30     virus ("AAV") vectors are also used to transduce cells with target nucleic acids, e.g., in the *in*

*vitro* production of nucleic acids and peptides, and for *in vivo* and *ex vivo* gene therapy

procedures (*see, e.g.,* West *et al., Virology* 160:38-47 (1987); U.S. Patent No. 4,797,368; WO

93/24641; Kotin, *Human Gene Therapy* 5:793-801 (1994); Muzyczka, *J. Clin. Invest.*

94:1351 (1994)). Construction of recombinant AAV vectors is described in a number of

publications, including U.S. Pat. No. 5,173,414; Tratschin *et al.*, *Mol. Cell. Biol.* 5:3251-3260 (1985); Tratschin, *et al.*, *Mol. Cell. Biol.* 4:2072-2081 (1984); Hermonat & Muzyczka, *PNAS* 81:6466-6470 (1984); and Samulski *et al.*, *J. Virol.* 63:03822-3828 (1989).

[191]    pLASN and MFG-S are examples are retroviral vectors that have been used in clinical trials (Dunbar *et al.*, *Blood* 85:3048-305 (1995); Kohn *et al.*, *Nat. Med.* 1:1017-102 (1995); Malech *et al.*, *PNAS* 94:22 12133-12138 (1997)). PA317/pLASN was the first therapeutic vector used in a gene therapy trial. (Blaese *et al.*, *Science* 270:475-480 (1995)). Transduction efficiencies of 50% or greater have been observed for MFG-S packaged vectors. (Ellem *et al.*, *Immunol Immunother.* 44(1):10-20 (1997); Dranoff *et al.*, *Hum. Gene Ther.* 1:111-2 (1997).

[192]    Recombinant adeno-associated virus vectors (rAAV) are a promising alternative gene delivery systems based on the defective and nonpathogenic parvovirus adeno-associated type 2 virus. All vectors are derived from a plasmid that retains only the AAV 145 bp inverted terminal repeats flanking the transgene expression cassette. Efficient gene transfer and stable transgene delivery due to integration into the genomes of the transduced cell are key features for this vector system. (Wagner *et al.*, *Lancet* 351:9117 1702-3 (1998), Kearns *et al.*, *Gene Ther.* 9:748-55 (1996)).

[193]    Replication-deficient recombinant adenoviral vectors (Ad) can be engineered such that a transgene replaces the Ad E1a, E1b, and E3 genes; subsequently the replication defector vector is propagated in human 293 cells that supply deleted gene function in trans. Ad vectors can transduce multiply types of tissues *in vivo*, including nondividing, differentiated cells such as those found in the liver, kidney and muscle system tissues. Conventional Ad vectors have a large carrying capacity. An example of the use of an Ad vector in a clinical trial involved polynucleotide therapy for antitumor immunization with intramuscular injection (Sterman *et al.*, *Hum. Gene Ther.* 7:1083-9 (1998)). Additional examples of the use of adenovirus vectors for gene transfer in clinical trials include Rosenecker *et al.*, *Infection* 24:1 5-10 (1996); Sterman *et al.*, *Hum. Gene Ther.* 9:7 1083-1089 (1998); Welsh *et al.*, *Hum. Gene Ther.* 2:205-18 (1995); Alvarez *et al.*, *Hum. Gene Ther.* 5:597-613 (1997); Topf *et al.*, *Gene Ther.* 5:507-513 (1998); Sterman *et al.*, *Hum. Gene Ther.* 7:1083-1089 (1998).

[194]    Packaging cells are used to form virus particles that are capable of infecting a host cell. Such cells include 293 cells, which package adenovirus, and ψ2 cells or PA317 cells, which package retrovirus. Viral vectors used in gene therapy are usually generated by producer cell line that packages a nucleic acid vector into a viral particle. The

vectors typically contain the minimal viral sequences required for packaging and subsequent integration into a host, other viral sequences being replaced by an expression cassette for the protein to be expressed. The missing viral functions are supplied in *trans* by the packaging cell line. For example, AAV vectors used in gene therapy typically only possess ITR sequences from the AAV genome which are required for packaging and integration into the host genome. Viral DNA is packaged in a cell line, which contains a helper plasmid encoding the other AAV genes, namely *rep* and *cap*, but lacking ITR sequences. The cell line is also infected with adenovirus as a helper. The helper virus promotes replication of the AAV vector and expression of AAV genes from the helper plasmid. The helper plasmid is not packaged in significant amounts due to a lack of ITR sequences. Contamination with adenovirus can be reduced by, e.g., heat treatment to which adenovirus is more sensitive than AAV.

[195]   In many gene therapy applications, it is desirable that the gene therapy vector be delivered with a high degree of specificity to a particular tissue type. A viral vector is typically modified to have specificity for a given cell type by expressing a ligand as a fusion protein with a viral coat protein on the viruses outer surface. The ligand is chosen to have affinity for a receptor known to be present on the cell type of interest. For example, Han *et al., PNAS* 92:9747-9751 (1995), reported that Moloney murine leukemia virus can be modified to express human heregulin fused to gp70, and the recombinant virus infects certain human breast cancer cells expressing human epidermal growth factor receptor. This principle can be extended to other pairs of virus expressing a ligand fusion protein and target cell expressing a receptor. For example, filamentous phage can be engineered to display antibody fragments (e.g., FAB or Fv) having specific binding affinity for virtually any chosen cellular receptor. Although the above description applies primarily to viral vectors, the same principles can be applied to nonviral vectors. Such vectors can be engineered to contain specific uptake sequences thought to favor uptake by specific target cells.

[196]   Gene therapy vectors can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (e.g., intravenous, intraperitoneal, intramuscular, subdermal, or intracranial infusion) or topical application, as described below. Alternatively, vectors can be delivered to cells *ex vivo*, such as cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the vector.

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[197]   *Ex vivo* cell transfection for diagnostics, research, or for gene therapy

(e.g., via re-infusion of the transfected cells into the host organism) is well known to those of

skill in the art. In some embodiments, cells are isolated from the subject organism,

transfected with a nucleic acid (gene or cDNA) encoding a polypeptides of the invention, and

5    re-infused back into the subject organism (e.g., patient). Various cell types suitable for *ex*

*vivo* transfection are well known to those of skill in the art (*see, e.g.,* Freshney *et al., Culture*

*of Animal Cells, A Manual of Basic Technique* (3rd ed. 1994)) and the references cited

therein for a discussion of how to isolate and culture cells from patients).

[198]   In one embodiment, stem cells are used in *ex vivo* procedures for cell

10   transfection and gene therapy. The advantage to using stem cells is that they can be

differentiated into other cell types *in vitro*, or can be introduced into a mammal (such as the

donor of the cells) where they will engraft in the bone marrow. Methods for differentiating

CD34+ cells *in vitro* into clinically important immune cell types using cytokines such a GM-

CSF, IFN-$\gamma$ and TNF-$\alpha$ are known (*see* Inaba *et al., J. Exp. Med.* 176:1693-1702 (1992)).

15   [199]   Stem cells are isolated for transduction and differentiation using

known methods. For example, stem cells are isolated from bone marrow cells by panning the

bone marrow cells with antibodies which bind unwanted cells, such as CD4+ and CD8+ (T

cells), CD45+ (panB cells), GR-1 (granulocytes), and Iad (differentiated antigen presenting

cells) (*see* Inaba *et al., J. Exp. Med.* 176:1693-1702 (1992)).

20   [200]   Vectors (e.g., retroviruses, adenoviruses, liposomes, etc.) containing

therapeutic nucleic acids can be also administered directly to the organism for transduction of

cells *in vivo*. Alternatively, naked DNA can be administered. Administration is by any of the

routes normally used for introducing a molecule into ultimate contact with blood or tissue

cells. Suitable methods of administering such nucleic acids are available and well known to

25   those of skill in the art, and, although more than one route can be used to administer a

particular composition, a particular route can often provide a more immediate and more

effective reaction than another route.

[201]   Pharmaceutically acceptable carriers are determined in part by the

particular composition being administered, as well as by the particular method used to

30   administer the composition. Accordingly, there is a wide variety of suitable formulations of

pharmaceutical compositions of the present invention, as described below (*see, e.g.,*

*Remington's Pharmaceutical Sciences*, 17th ed., 1989).

## X.    DIAGNOSIS OF DIABETES

[202]    The present invention also provides methods of diagnosing diabetes or a predisposition of at least some of the pathologies of diabetes. Diagnosis can involve determination of a genotype of an individual (e.g., with SNPs) and comparison of the genotype with alleles known to have an association with the occurrence of diabetes. Alternatively, diagnosis also involves determining the level of a polypeptide or polynucleotide of the invention in a patient and then comparing the level to a baseline or range. Typically, the baseline value is representative of a polypeptide or polynucleotide of the invention in a healthy (e.g., lean) person.

[203]    As discussed above, variation of levels (e.g., low or high levels) of a polypeptide or polynucleotide of the invention compared to the baseline range indicates that the patient is either diabetic or at risk of developing at least some of the pathologies of diabetes (e.g., pre-diabetic). The level of a polypeptide in a lean individual can be a reading from a single individual, but is typically a statistically relevant average from a group of lean individuals. The level of a polypeptide in a lean individual can be represented by a value, for example in a computer program.

[204]    In some embodiments, the level of polypeptide or polynucleotide of the invention is measured by taking a blood, urine or tissue sample from a patient and measuring the amount of a polypeptide or polynucleotide of the invention in the sample using any number of detection methods, such as those discussed herein. For instance, fasting and fed blood or urine levels can be tested.

[205]    In some embodiments, the baseline level and the level in a lean sample from an individual, or at least two samples from the same individual differ by at least about 5%, 10%, 20%, 50%, 75%, 100%, 150%, 200%, 300%, 400%, 500%, 1000% or more. In some embodiments, the sample from the individual is greater by at least one of the above-listed percentages relative to the baseline level. In some embodiments, the sample from the individual is lower by at least one of the above-listed percentages relative to the baseline level.

[206]    In some embodiments, the level of a polypeptide or polynucleotide of the invention is used to monitor the effectiveness of antidiabetic therapies such as thiazolidinediones, metformin, sulfonylureas and other standard therapies. In some embodiments the activity or expression of a polypeptide or polynucleotide of the invention will be measured prior to and after treatment of diabetic or pre-diabetic patients with antidiabetic therapies as a surrogate marker of clinical effectiveness. For example, the

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greater the reduction in expression or activity of a polypeptide of the invention indicates greater effectiveness.

[207]   Glucose/insulin tolerance tests can also be used to detect the effect of glucose levels on levels of a polypeptide or polynucleotide of the invention. In glucose tolerance tests, the patient's ability to tolerate a standard oral glucose load is evaluated by assessing serum and urine specimens for glucose levels. Blood samples are taken before the glucose is ingested, glucose is given by mouth, and blood or urine glucose levels are tested at set intervals after glucose ingestion. Similarly, meal tolerance tests can also be used to detect the effect of insulin or food, respectively, on levels of a polypeptide or polynucleotide of the invention.

[208]   All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

[209]   Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

EXAMPLES

[210]   The following examples are offered to illustrate, but not to limit the claimed invention.

[211]   The molecular mechanism by which thiazolidinediones (TZDs) cause an increase in peripheral insulin sensitivity was studied. Genes in muscle or fat whose expression is altered by TZDs may lie on a pathway leading from TZD treatment to increased insulin sensitivity. Modulators of such genes can elicit the same effect as TZD treatment. Moreover, such modulators can lack some of the side effects of TZD. The majority of glucose disposal occurs in muscle. For this reason, gene expression profiling in human muscle from diabetics treated with troglitazone was used to identify genes important for TZD action and therefore treatment of diabetes and insulin resistance.

[212]   Gene expression profiling was performed on tissue samples (muscle) obtained from lean obese and diabetic individuals. Two studies were performed. In the first study, basal samples were isolated from all individuals at the beginning of a 5 hour

hyperinsulinemic euglycemic clamp. Clamp samples were isolated at the end of this

procedure. Similar basal and clamp samples were taken 3 months later after all patients had

taken the insulin sensitizing drug troglitazone.

[213]   In the second study, samples were obtained from lean, obese and

diabetic individuals before and after a hyperinsulinemic euglycemic clamp. No troglitazone

treatment was used. For all tissue samples mRNA was isolated from these muscle samples

and converted to cRNA by standard procedures. The gene expression profile for each

individual was determined by hybridization of cRNA to custom synthesized Affymetrix

chips.

[214]   Gene expression profile differences were calculated as follows. The

expression level of a particular gene is indicated by its 'average difference score'. The raw

data was analyzed by a statistical test to remove 'outliers'. The mean 'average difference

score' was then calculated from the average difference scores for all individuals in a

particular treatment group. Genes were determined to be changed in condition 1 (such as

basal diabetics before trogliazone (tro) treatment) versus condition 2 (such as basal diabetics

after troglitazone treatment) by calculating the Students t test statistic between the two

conditions and selecting those with t less than or equal to 0.05. Fold change was determined

as the ratio of mean average difference score in condition 2 to the mean average difference

score in condition 1.

[215]   Probe set mbxhummus 24330 detects expression of connective tissue

growth factor (CTGF) mRNA.

[216]   Expression of transcripts encoding Connective Tissue Growth Factor

(SEQ ID NO:2) was decreased in tro-treated patients compared to untreated patients in this

study.

:

|  | Diabetic Pre-Trog | | | Diabetic Post-Trog | | | | | |
|-----|--------------|-----|-----|--------------|-----|-----|-----------------|------------------|-----------|
| B/C | Mean Expr | SEM | n | Mean Expr | SEM | n | Fold Change | Students t test | Gene name |
| C | 409 | 83 | 9 | 29 | 5 | 10 | 0.07 | 0.016 | Connective Tissue Growth Factor |

Legend: B/C indicates sample is from Basal or Clamp; "Pre-Trog" and "Post-Trog" indicates

sample was taken before or after 3 months of Troglitazone treatment; "Mean Expr" indicates

mean expression; "SEM" indicates standard error of mean; "n" indicates number of patient

samples; "Fold Change" indicates fold change of diabetic post tro/diabetic pre tro.

[217]    Connective Tissue Growth Factor (SEQ ID NO:2) contains the

following protein domains:

Signal peptide at amino acids 1 to 27;
Insulin growth factor-binding protein homologues domain at amino acids 27 to 97;
5    Von Willebrand factor (vWF) type C domain at amino acids 103 to 166;
Thrombospondin type 1 repeat Domain at amino acids 200 to 243; and
C-terminal cystine knot-like domain at amino acids 261 to 330.

[218]    Probe set mbxhummus23808 detects expression of TIEG mRNA

10   [219]    Expression of transcripts encoding TIEG (SEQ ID NO:8) was higher in

diabetic patients compared to lean, non-diabetic patients in this study.

|       | Lean Pre-Trog |     |    | Diabetic Pre-Trog |     |    |        |          |       |
|-------|---------------|-----|----|-------------------|-----|----|--------|----------|-------|
| B/C   | Mean Expr     | SEM | n  | Mean Expr         | SEM | n  | Fold Change | Students t test | Gene name |
| B     | 1593          | 161 | 17 | 3335              | 249 | 19 | 2.094  | 0.000    | TIEG  |

[220]    Expression of transcripts encoding TIEG (SEQ ID NO:8) was reduced

in tro-treated patients compared to untreated patients.

|       | Diabetic Pre-Trog |     |    | Diabetic Post-Trog |     |    |        |          |       |
|-------|-------------------|-----|----|--------------------|-----|----|--------|----------|-------|
| B/C   | Mean Expr         | SEM | n  | Mean Expr          | SEM | n  | Fold Change | Students t test | Gene name |
| B     | 4061              | 559 | 9  | 2634               | 285 | 10 | 0.65   | 0.022    | TIEG  |

15

**Legend:** B/C indicates sample is from Basal or Clamp; "Pre-Trog" and "Post-Trog" indicates

sample was taken before or after 3 months of Troglitazone treatment; "Mean Expr" indicates

mean expression; "SEM" indicates standard error of mean; "n" indicates number of patient

samples; "Fold Change" indicates fold change of diabetic post-trog/diabetic pre-trog.

20   [221]    Transcripts encoding TIEG (SEQ ID NO:8) contain the following

protein domains:

zf-C2H2 domain encoded by amino acids 358 to 382

zf-C2H2 domain encoded by amino acids 388 to 412

zf-C2H2 domain encoded by amino acids 418 to 440.

25   [222]    A splice variant of TIEG is also known (SEQ ID NO:13), which

encodes the amino acid sequence displayed in SEQ ID NO:14.

[223]    Transcripts encoding TIEG (SEQ ID NO:13) contain the following

protein domains:

zf-C2H2 domain encoded by amino acids 369 to 393

30   zf-C2H2 domain encoded by amino acids 399 to 423

zf-C2H2 domain encoded by amino acids 429 to 440.

[224]   The gene expression changes observed in the human muscle samples derived from lean and diabetic individuals or from diabetic individuals post-Troglitazone treatment, were further confirmed by the use of real-time quantitative PCR. A combination of specific PCR primers and a Taqman probe were designed to detect and quantify expression levels for each gene. Relative gene expression levels were determined in this manner in each patient and the mean expression level in either the lean or diabetic patient population was then calculated.

[225]   In order to demonstrate the ability of the gene to modulate insulin sensitivity a number of different analyses were undertaken. One analysis called "Glut 4 translocation" involves over-expressing the gene in 3T3-L1 adipocytes and then monitoring the ability of insulin to cause the movement (translocation) of the major insulin-regulated glucose transporter, Glut 4, to the cell surface. The cDNA encoding the human gene was sub-cloned into the mammalian expression vector pcDNA3.1 which provides for a V5 epitope tag. This plasmid encoding the human gene was then introduced into differentiated murine 3T3-L1 adipocytes along with a cloned Glut 4 expression construct using an electroporation procedure essentially as described in Kanzaki *et al. J. Biol.Chem* 275 7167-7175 (2000). A plasmid expressing LacZ was used as a negative control. After stimulation with increasing concentrations of insulin, cells co-expressing both the human gene and cloned Glut 4 were scored for the presence of Glut 4 at the cell surface using fluorescence microscopy.

[226]   In some instances another analysis called "glucose transport" was used. This involves over-expressing the gene in either differentiated 3T3-L1 adipocytes or differentiated L6 myotubes and then monitoring the ability of insulin to stimulate glucose transport into these two cell types. A recombinant adenovirus encoding the human gene tagged with a FLAG epitope tag was prepared essentially as described in Zhou *et al. Proc. Nat. Acad. Sci. USA.* 95: 2509-2514. 3T3-L1 adipocytes or L6 myotubes were infected with recombinant adenovirus expressing the human gene. A virus expressing an irrelevant protein was used as the control. 24 h post-infection the cells are stimulated with increasing concentrations of insulin and glucose transport into the cells determined by the counting the accumulation of a radiolabelled glucose analog (2-deoxyglucose) essentially as describe in Kotani *et al. Mol. Cell. Biol.* 18 6971-6982 (1998), Fujishiro *et al. J. Biol. Chem.* 276 19800-19806 (2001), Ross *et al. Biochem. Biophys. Res. Commun.* 302 354-358 (2003).

## A.    Connective Tissue Growth Factor

**Real time PCR analysis**

| Comparison | Expression Fold change | t test |
|---|---|---|
| Diabetics + Tro (10) /Diabetics - Tro  (10) | 0.35 | 0.042 |

5    **Legend** "Tro" indicates treatment with Troglitazone. "Fold change" indicates fold change in CTGF expression calculated as the ratio of mean diabetic post-Tro expression/mean diabetic pre-Tro expression. Numbers in parentheses indicate the number of patient samples analyzed by real time PCR.

10    [227]   These data further show that treatment with Troglitazone reduces the expression of CTGF in muscle from diabetic individuals.

**Glut 4 translocation analysis**

| Insulin (nM) | Fold Change (Mean hCTGF/Mean LacZ) (n=3) | t test (hCTGF vs LacZ) |
|---|---|---|
| 0 | 0.97 | 0.960 |
| 1 | 0.35 | 0.044 |
| 10 | 0.45 | 0.010 |

**Legend** "Fold Change" indicates the following ratio; (Mean % of CTGF-expressing cells scored positive for cell surface Glut 4)/(Mean % of LacZ-expressing cells scored positive for
15    cell surface Glut 4). "n" is the number of replicates.

[228]   These data show that increasing the levels of hCTGFin 3T3-L1 adipocytes significantly inhibited Glut 4 translocation at all insulin concentrations tested with little or no effect on basal Glut 4 translocation.

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**Glucose transport analysis in 3T3-L1 adipocytes**

| Insulin (nM) | Expt 1 | | Expt 2 | | Expt 3 | | Mean FC (hCTGF/Con) +/- SEM (n=3) |
|---|---|---|---|---|---|---|---|
| | FC (hCTGF/Con) | t test | FC (hCTGF/Con) | t test | FC (hCTGF/Con) | t test | |
| 0 | 0.7 | 0.056 | 0.6 | 0.085 | 0.8 | 0.116 | 0.70 +/- 0.03 |
| 0.05 | 0.6 | 0.076 | 0.6 | 2.11E-04 | 0.7 | 0.233 | 0.63 +/- 0.06 |
| 0.1 | 0.6 | 0.038 | 0.7 | 0.120 | 0.7 | 0.183 | 0.67 +/- 0.02 |
| 0.3 | 0.7 | 0.057 | 0.8 | 0.130 | 0.8 | 0.395 | 0.76 +/- 0.05 |
| 1 | 0.8 | 0.027 | 0.8 | 0.180 | 0.9 | 0.308 | 0.83 +/- 0.02 |
| 10 | 0.6 | 0.015 | 0.7 | 0.012 | 0.9 | 0.143 | 0.75 +/- 0.10 |

**Legend** "Con" indicates control cells which do not express hCTGF. "FC" indicates the fold change defined as the following ratio; glucose transport in hCTGF-expressing cells/glucose transport in non-hCTGF-expressing cells. h" is human. "n" is the number of replicates. SEM
25    is the standard error of the mean.

[229]   These data show that increasing the levels of hCTGF in 3T3-L1

adipocytes inhibited both basal and insulin stimulated glucose transport.

**Glucose transport analysis in L6 myotubes**

| Insulin (nM) | Expt 1 | | Expt 2 | | Expt 3 | | Mean FC (CTGF/Con) +/- SEM (n=3) |
|---|---|---|---|---|---|---|---|
| | FC (hCTGF/Con) | t test | FC (hCTGF/Con) | t test | FC (hCTGF/Con) | t test | |
| 0 | 0.78 | 2.49E-04 | 0.79 | 0.042 | 0.79 | 3.6E-05 | 0.79 +/- 0.004 |
| 10 | 0.67 | 0.163 | 0.92 | 0.121 | 0.77 | 0.080 | 0.79 +/- 0.07 |
| 100 | 0.70 | 0.226 | 1.05 | 0.404 | 0.74 | 0.001 | 0.83 +/- 0.11 |

**Legend** "Con" indicates control cells which do not express CTGF. "FC" indicates the fold
change defined as the following ratio; glucose transport in CTGF-expressing cells/glucose
transport in non-CTGF-expressing cells. h" is human. "n" is the number of replicates. SEM
is the standard error of the mean.

[230]   These data show that increasing the levels of hCTGF in L6 myotubes

inhibited both basal and insulin stimulated glucose transport.  These data are consistent with

the data obtained in 3T3-L1 adipocytes.

[231]   Decreased CTGF levels such as those observed in diabetic patients

treated with troglitazone would therefore be beneficial at improving glucose uptake into

muscle and fat.

**B.      TIEG**

**Real time PCR analysis**

| Comparison | Expression Fold change | t test |
|---|---|---|
| Diabetics + Tro (9) /Diabetics - Tro (9) | 0.69 | 0.057 |
| Diabetics (19)/Lean (17) | 2.56 | 0.005 |

**Legend** "Tro" indicates treatment with Troglitazone. "Fold change" indicates fold change in
TIEG expression calculated as either the ratio of mean diabetic post-Tro expression/mean
diabetic pre-Tro expression or mean diabetic pre-Tro expression/mean lean pre-Tro
expression.  Numbers in parentheses indicate the number of patient samples analyzed by real
time PCR.

[232]   These data further show that TIEG is significantly over-expressed in

muscle from diabetic individuals when compared to muscle from lean individuals.

Furthermore the data also shows that treatment with Troglitazone reduces the expression of

TIEG in muscle from diabetic individuals.

**Glut 4 translocation analysis**

| Insulin (nM) | Fold Change (Mean hTIEG/Mean LacZ) (n=3) | t test (hTIEG vs LacZ) |
|---|---|---|
| 0 | 1.34 | 0.600 |
| 1 | 0.20 | 0.019 |
| 10 | 0.11 | 5.1E-05 |

**Legend** "Fold Change" indicates the following ratio; (Mean % of hTIEG-expressing cells scored positive for cell surface Glut 4)/(Mean % of LacZ-expressing cells scored positive for cell surface Glut 4). h" is human. "n" is the number of replicates.

[233] These data show that increasing the levels of hTIEG in 3T3-L1 adipocytes significantly inhibited Glut 4 translocation at all insulin concentrations tested with little or no effect on basal Glut 4 translocation. Increased TIEG levels such as those observed in human diabetic muscle are therefore inhibitory to insulin action.

<u>WHAT IS CLAIMED IS:</u>

1        1.        A method for identifying an agent for treating a diabetic or pre-diabetic

2    individual, the method comprising the steps of:

3              (i)        contacting an agent to a mixture comprising a polypeptide encoded by

4    a polynucleotide that hybridizes under stringent conditions to a nucleic acid encoding SEQ

5    ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or

6    SEQ ID NO:14; and

7              (ii)        selecting an agent that modulates the expression or activity of the

8    polypeptide or that binds to the polypeptide, thereby identifying an agent for treating a

9    diabetic or pre-diabetic individual.

1        2.        The method of claim 1, the method further comprising selecting an

2    agent that modulates insulin sensitivity.

1        3.        The method of claim 1, wherein step (ii) comprises selecting an agent

2    that modulates expression of the polypeptide.

1        4.        The method of claim 1, wherein step (ii) comprises selecting an agent

2    that modulates the activity of the polypeptide.

1        5.        The method of claim 1, wherein step (ii) comprises selecting an agent

2    that specifically binds to the polypeptide.

1        6.        The method of claim 1, wherein the polypeptide is expressed in a cell

2    and the cell is contacted with the agent.

1        7.        A method of treating a diabetic or pre-diabetic animal, the method

2    comprising administering to the animal a therapeutically effective amount of an agent

3    identified by the method of claim 1.

1        8.        The method of claim 7, wherein the agent is an antibody.

1        9.        The method of claim 8, wherein the antibody is a monoclonal

2    antibody.

1        10.        The method of claim 7, wherein the animal is a human.

1   11.   A method of introducing an expression cassette into a cell, the method

2   comprising,

3   introducing into the cell an expression cassette comprising a promoter

4   operably linked to a polynucleotide encoding a polypeptide, wherein the polynucleotide

5   hybridizes under stringent conditions to a nucleic acid encoding SEQ ID NO:2, SEQ ID

6   NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12 or SEQ ID NO:14.


1   12.   The method of claim 11, wherein the cell is selected from the group

2   consisting of an adipocyte and a skeletal muscle cell.


1   13.   The method of claim 11, the method further comprising introducing

2   the cell into a human.


1   14.   The method of claim 13, wherein the human is diabetic.


1   15.   The method of claim 13, wherein the human is prediabetic.


1   16.   The method of claim 13, wherein the cell is from the human.


1   17.   A method of diagnosing an individual who has Type 2 diabetes or is

2   prediabetic, the method comprising,

3   detecting in a sample from the individual the level of a polypeptide or the level

4   of a polynucleotide encoding the polypeptide, wherein the polynucleotide hybridizes under

5   stringent conditions to a nucleic acid encoding an amino acid sequence selected from the

6   group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID

7   NO:10, SEQ ID NO:12 or SEQ ID NO:14,

8   wherein a modulated level of the polypeptide or polynucleotide in the sample

9   compared to a level of the polypeptide or polynucleotide in either a lean individual or a

10  previous sample from the individual indicates that the individual is diabetic or prediabetic.


1   18.   The method of claim 17, wherein the detecting step comprises

2   contacting the sample with an antibody that specifically binds to the polypeptide.


1   19.   The method of claim 17, wherein the detecting step comprises

2   quantifying mRNA encoding the polypeptide.

1          20.     The method of claim 19, wherein the mRNA is reverse transcribed and

2     amplified in a polymerase chain reaction.

1          21.     The method of claim 17, wherein the sample is a blood, urine or tissue

2     sample.

1          22.     An isolated nucleic acid encoding SEQ ID NO:14.

1          23.     The isolated nucleic acid of claim 22, wherein the nucleic acid

2     comprises SEQ ID NO:13.

1          24.     An expression cassette comprising a heterologous promoter operably

2     linked to a polynucleotide encoding SEQ ID NO:14

1          25.     The expression cassette of claim 24, wherein the polynucleotide

2     comprises SEQ ID NO:13.

1          26.     A host cell comprising the expression cassette of claim 24.

SEQUENCE LISTING

## SEQ ID NO:1 Human CTGF nucleic acid

```
>Nucleotide sequence    HUM205074    accession:X78947   CDS:146..1195
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CGCCGCCAGTATGGGCCCCGTCCGCGTCGCCTTCGTGGTCCTCCTCGCCCTCTGCAGCCGGCCGGCCGTCGGCCA
GAACTGCAGCGGGCCGTGCCGGTGCCCGGACGAGCCGGCGCCGCGCTGCCCGGCGGGCGTGAGCCTCGTGCTGGA
CGGCTGCGGCTGCTGCCGCGTCTGCGCCAAGCAGCTGGGCGAGCTGTGCACCGAGCGCGACCCCTGCGACCCGCA
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```

## SEQ ID NO:2 Human CTGF polypeptide

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>Protein sequence protein_id:gi474934
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PRRVKLPGKCCEEWVCDEPKDQTVVGPALAAYRLEDTFGPDPTMIRANCLVQTTEWSACSKTCGMGISTRVTNDN
ASCRLEKQSRLCMVRPCEADLEENIKKGKKCIRTPKISKPIKFELSGCTSMKTYRAKFCGVCTDGRCCTPHRTTT
LPVEFKCPDGEVMKKNMMFIKTCACHYNCPGDNDIFESLYYRKMYGDMA
```

## SEQ ID NO:3

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MOUSE CTGF HOMOLOG:
>Nucleotide sequence    accession:M80263                CDS:204..1250
AGACTCAGCCAGATCCACTCCAGCTCCGACCCCAGGAGACCGACCTCCTCCAGACGGCAGCAGCCCCAGCCCAGC
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CGCCTCTCGTCGCCTCTGCACCCTGCTGTGCATCCTCCTACCGCGTCCCGATCATGCTCGCCTCCGTCGCAGGTC
CCATCAGCCTCGCCTTGGTGCTCCTCGCCCTCTGCACCCGGCCTGCTACGGGCCAGGACTGCAGCGCGCAATGTC
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TGGAAGACACATTTGGCCCAGACCCAACTATGATGCGAGCCAACTGCCTGGTCAGACCACAGAGTGGAGCGCCT
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AAAAA


## SEQ ID NO:4 Mouse CTGF polypeptide

>Protein sequence accession:gi109590
MLASVAGPISLALVLLALCTRPATGQDCSAQCQCAAEAAPHCPAGVSLVLDGCGCCRVCAKQLGELCTERDPCDP
HKGLFCDFGSPANRKIGVCTAKDGAPCVFGGSVYRSGESFQSSCKYQCTCLDGAVGCVPLCSMDVRLPSPDCPFP
RRVKLPGKCCEEWVCDEPKDRTAVGPALAAYRLEDTFGPDPTMMRANCLVQTTEWSACSKTCGMGISTRVTNDNT
FCRLEKQSRLCMVRPCEADLEENIKKGKKCIRTPKIAKPVKFELSGCTSVKTYRAKFCGVCTDGRCCTPHRTTTL
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## SEQ ID NO:5

RAT CTGF HOMOLOG:
>Nucleotide sequence        accession:NM_022266              CDS:225..1268
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## SEQ ID NO:6 Rat CTGF polypeptide

>Protein sequence accession:gi|1560085
MLASVAGPVSLALVLLLCTRPATGQDCSAQCQCAAEAAPRCPAGVSLVLDGCGCCRVCAKQLGELCTERDPCDPH
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RVKLPGKCCEEWVCDEPKDRTVVGPALAAYRLEDTFGPDPTMMRANCLVQTTEWSACSKTCGMGISTRVTNDNTF
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## SEQ ID NO:7 TIEG Human nucleic acid

>Nucleotide sequence       HUM202520       accession:BC011538       CDS:320..1729
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## SEQ ID NO:8 Human TIEG polypeptide

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>Protein sequence protein_id:gi15079389
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AQLCNHQTCPMKAASILNYQNNSFRRRTHLNVEAARKNIPCAAVSPNRSKCERNTVADVDEKASAALYDFSVPSS
ETVICRSQPAPVSPQQKSVLVSPPAVSAGGVPPMPVICQMVPLPANNPVVTTVVPSTPPSQPPAVCPPVVFMGTQ
VPKGAVMFVVPQPVVQSSKPPVVSPNGTRLSPIAPAPGFSPSAAKVTPQIDSSRIRSHICSHPGCGKTYFKSSHL
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## SEQ ID NO:9

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TIEG MOUSE HOMOLOG:
>Nucleotide sequence        accession:NM_013692               CDS:114..1553
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## SEQ ID NO:10 TIEG Mouse polypeptide

>Protein sequence accession:gi7305571
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## SEQ ID NO:11 TIEG Rat polynucleotide

RAT HOMOLOG:
>Nucleotide sequence        accession:NM_031135                    CDS:316..1758
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SEQ ID NO:12 Rat polypeptide

>Protein sequence accession:gi13592117
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SEQ ID NO:13 Human TIEG splice variant

>Nucleotide sequence                    accession:U21847      CDS:87..1529
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AGTTTTAATTAAAAAAAATCCTTGTGACTTACAATTTGCACAATATTTCTTTTGTTGTACTTTATATCTTGTTTA
CAATAAAGAATTCCCTTTGGCA


SEQ ID NO:14 Polypeptide encoded by human TIEG splice variant

>Protein sequence protein_id:gi1155215
            MLNFGASLQQTAEERMEMISERPKESMYSWNKTAEKSDFEAVEALMSMSCSWKSDFKKYVENR
PVTPVSDLSEEENLLPGTPDFHTIPAFCLTPPYSPSDFEPSQVSNLMAPAPSTVHFKSLSDTAKPHIAAPFKEEE
KSPVSAPKLPKAQATSVIRHTADAQLCNHQTCPMKAASILNYQNNSFRRRTHLNVEAARKNIPCAAVSPNRSKCE

RNTVADVDEKASAALYDFSVPSSETVICRSQPAPVSPQQKSVLVSPPAVSA...PPMPVICQMVPLPANNPVVTT
VVPSTPPSQPPAVCPPVVFMGTQVPKGAVMFVVPQPVVQSSKPPVVSPNGTRLSPIAPAPGFSPSAAKVTPQIDS
SRIRSHICSHPGCGKTYFKSSHLKAHTRTHTGEKPFSCSWKGCERRFARSDELSRHRRTHTGEKKFACPMCDRRF
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